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(54) Title: IL-8 ANTAGONISTS FOR TREATMENT OF INFLAMMATORY DISORDERS AND ASTHMA					
(57) Abstract					
Methods are provided for the treatment of asthma with IL-8 antagonists.					

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**IL-8 ANTAGONISTS
FOR TREATMENT OF INFLAMMATORY DISORDERS AND ASTHMA**

FIELD OF THE INVENTION

5 This application relates to IL-8 antagonists, such as anti-interleukin-8 (IL-8) antibodies, and their use in the treatment of inflammatory disorders and asthma.

BACKGROUND

10 Interleukin-8 (IL-8) is neutrophil chemotactic peptide secreted by a variety of cells in response to inflammatory mediators (for a review see Hebert et al. *Cancer Investigation* 11(6):743 (1993)). IL-8 can play an important role in the pathogenesis of inflammatory disorders, such as adult respiratory distress syndrome (ARDS), septic shock, and multiple organ failure. Immune therapy for such inflammatory disorders can include treatment of an affected patient with anti-IL-8 antibodies.

15 Sticherling et al. (*J. Immunol.* 143:1628 (1989)) disclose the production and characterization of four monoclonal antibodies against IL-8. WO 92/04372, published March 19, 1992, discloses polyclonal antibodies which react with the receptor-interacting site of IL-8 and peptide analogs of IL-8, along with the use of such antibodies to prevent an inflammatory response in patients. St. John et al. (*Chest* 103:932 (1993)) review immune therapy for ARDS, septic shock, and multiple organ failure, including the potential therapeutic use of anti-IL-8 antibodies. Sekido et al. (*Nature* 365:654 (1993)) disclose the prevention of lung reperfusion injury in rabbits by a monoclonal antibody against IL-8. Mulligan et al. (*J. Immunol.* 150:5585 (1993)), disclose protective effects of a murine monoclonal antibody to human IL-8 in inflammatory lung injury in rats.

20 The instant invention demonstrates that the anti-IL-8 monoclonal antibodies of the invention can be used therapeutically in the treatment of other inflammatory disorders, such as bacterial pneumonias and inflammatory bowel disease.

25 Anti-IL-8 antibodies are additionally useful as reagents for assaying IL-8. For example, Sticherling et al. (*Arch. Dermatol. Res.* 284:82 (1992)), disclose the use of anti-IL-8 monoclonal antibodies as reagents in immunohistochemical studies. Ko et al. (*J. Immunol. Methods* 149:227 (1992)) disclose the use of anti-IL-8 monoclonal antibodies as reagents in an enzyme-linked immunoabsorbent assay (ELISA) for IL-8.

30 The invention further demonstrates that IL-8 antagonists, including anti-IL-8 monoclonal antibodies, can be used therapeutically in the treatment of asthma.

SUMMARY OF THE INVENTION

35 The invention provides a method of treating asthma in a subject comprising administering a therapeutically effective amount of an IL-8 antagonist. The methods of the invention provide for administration of IL-8 antagonist to the subject before and/or after the onset of asthma.

40 In one aspect, the invention provides a method of treating asthma with an anti-IL-8 antibody.

45 In another aspect, the invention provides a method of treating asthma with an IL-8 antagonist that inhibits IL-8 binding to neutrophils.

In still another aspect, the invention provides a method of treating asthma with an IL-8 antagonist that inhibits neutrophil chemotaxis induced by IL-8.

In a further aspect, the invention provides a method of treating asthma with an IL-8 antagonist that inhibits neutrophil elastase release induced by IL-8.

5

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a graph depicting the blocking of IL-8 mediated elastase release by neutrophils by anti-IL-8 monoclonal antibody 5.12.14.

Figure 2 is a graph depicting the inhibition of ^{125}I -IL-8 binding to neutrophils by unlabeled IL-8.

Figure 3 demonstrates a negative isotype matched Fab does not inhibit the binding of ^{125}I -IL-8 to 10 human neutrophils.

Figure 4 is a graph depicting the inhibition of binding of ^{125}I -IL-8 to human neutrophils by chimeric 5.12.14 Fab with an average IC_{50} of 1.6 nanomoles/liter (nM).

Figure 5 is a graph depicting the inhibition of binding of ^{125}I -IL-8 to human neutrophils by chimeric 6G4.25 Fab with an average concentration required to achieve 50% inhibition of binding (IC_{50}) of 7.5 nM.

Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab.

Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

Figure 8 depicts the stimulation of elastase release from human neutrophils by various concentrations 20 of human and rabbit IL-8. The relative extent of elastase release was quantitated by measurement of absorbance at a wavelength of 405 nanometers (nm). The data represent mean \pm standard error of the mean (SEM) of triplicate samples.

Figure 9 is a graph depicting the ability of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab to inhibit 25 elastase release from human neutrophils stimulated by human IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC_{50} values were calculated by four parameter fit.

Figure 10 is a graph depicting the relative abilities of chimeric 6G4.2.5 Fab and chimeric 5.12.14 Fab 30 to inhibit elastase release from human neutrophils stimulated by rabbit IL-8. The results were normalized to reflect the percentage of elastase release elicited by 100 nM IL-8 alone. The data represent the mean \pm SEM of three separate experiments performed on different days with different blood donors. IC_{50} values were calculated by four parameter fit.

Figure 11, parts a-j, is a set of graphs depicting the following parameters in a rabbit ulcerative colitis model: (a) myeloperoxidase levels in tissue; (b) IL-8 levels in tissue; (c) colon weight; (d) gross inflammation; 35 (e) edema; (f) extent of necrosis; (g) severity of necrosis; (h) neutrophil margination; (i) neutrophil infiltration; (j) mononuclear infiltration.

Figure 12 is a graph depicting the effect of anti-IL-8 monoclonal antibody treatment on the number of neutrophils in bronchoalveolar lavage (BAL) fluid in animals infected with Streptococcus pneumoniae,

Escherichia coli, or Pseudomonas aeruginosa. Treatment with 6G4.2.5 significantly reduced the number of neutrophils present in the BAL fluid compared to animals treated with isotype control mouse IgG (Figure 12).

Figure 13 depicts the DNA sequences of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 5.12.14.

Figure 14 depicts the DNA sequences of one forward primer and one reverse primer for the 5.12.14 light chain variable region amplification.

Figure 15 depicts the DNA sequences of one forward primer and one reverse primer for the 5.12.14 heavy chain variable region amplification.

Figure 16 depicts the DNA sequence of the 5.12.14 light chain variable region. Complementarity-determining regions (CDRs) are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The partial murine constant light region is amino acids 110 to 123 (in italics).

Figure 17 depicts the DNA sequence of the 5.12.14 heavy chain variable region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Important restriction sites are indicated in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The partial murine constant heavy region is amino acids 121 to 130.

Figure 18 depicts the DNA sequences of amplification primers used to convert murine light and heavy chain constant region residues to their human equivalents.

Figure 19 depicts the coding sequence for the 5.12.14 light chain variable region and the human IgG1 light chain constant region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 109. The human constant light region is amino acids 110 to 215.

Figure 20 depicts the coding sequence for the 5.12.14 heavy chain variable region and the heavy chain constant region of human IgG1. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 120. The human constant heavy region is amino acids 121 to 229.

Figure 21 depicts the DNA sequences of three primers designed for each of the light and heavy chains. Multiple primers were designed in order to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis for cloning the variable light and heavy regions of monoclonal antibody 6G4.2.5.

Figure 22 depicts the DNA sequences of one forward primer and one reverse primer for the 6G4.2.5 light chain variable region amplification.

Figure 23 depicts the DNA sequences of one forward primer and one reverse primer for the 6G4.2.5 heavy chain variable region amplification.

Figure 24 depicts the DNA sequence of the 6G4.2.5 light chain variable region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable light region is amino acids 1 to 114. The partial murine constant light region is amino acids 115 to 131.

Figure 25 depicts the DNA sequence of the 6G4.2.5 heavy chain variable region. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). Useful cloning sites are in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The partial murine constant heavy region is amino acids 123 to 135.

Figure 26 depicts primers to convert the murine light chain and heavy chain constant regions to their human equivalents.

Figure 27 depicts the coding sequence for the chimeric 6G4.2.5 light chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 114. The human constant heavy region is amino acids 115 to 220.

Figure 28 depicts the coding sequence for the chimeric 6G4.2.5 heavy chain. CDRs are indicated by either X-ray crystallography (underlined amino acids) or by Kabat sequence comparison (amino acids denoted with asterisk). The human constant region is denoted in italics. The signal peptide of STII is amino acids -23 to -1. The murine variable heavy region is amino acids 1 to 122. The human constant heavy region is amino acids 123 to 231.

Figure 29 is a graph depicting the effect of the absence of IL-8 receptor homolog (IL8Rh) on eosinophil, macrophage, lymphocyte and neutrophil transmigration into the lung in an asthma model using wild type and IL8Rh knock-out (KO) mice. Transmigration levels are presented as bronchoalveolar lavage (BAL) fluid cell counts. Cell counts for wild type (WT) and IL8Rh KO control mice that were exposed to aerosolized ovalbumin allergen without prior allergen challenge are denoted as "WT OVA aero" and "KO OVA aero", respectively. Cell counts for wild type and IL8Rh KO mice that were intraperitoneally inoculated with ovalbumin allergen and subsequently exposed to aerosolized allergen are denoted as "WT OVA ip/aero" and "KO OVA ip/aero", respectively. Eosinophil, macrophage, lymphocyte and neutrophil cell counts are depicted with diagonally hatched, hatched, vertically striped, and solid columns, respectively.

Figure 30 is a graph depicting the effect of the absence of IL8Rh on peripheral eosinophil counts in an asthma model using wild type and IL8Rh knock-out mice. Peripheral eosinophil levels are presented as circulating blood eosinophil cell counts. Cell counts for wild type and KO mice that were intraperitoneally (ip) inoculated with ovalbumin allergen and subsequently exposed to aerosolized allergen are denoted as "WT" and "KO", respectively. Cell counts obtained on day 0 (before ip inoculation of allergen) are depicted as diagonally hatched columns. Cell counts obtained on day 21 (the day following completion of aerosolized allergen challenge) are depicted as solid columns.

Figure 31 is a graph depicting the effect of the absence of IL8Rh on eosinophil transmigration into the lung and peripheral eosinophil proliferation in an asthma model using wild type and IL8Rh knock-out (KO) mice. Eosinophil transmigration is presented as BAL fluid eosinophil counts (diagonally hatched columns). Circulating blood eosinophil counts are shown as solid columns. Cell counts obtained on day 0 before ip 5 allergen inoculation of WT and IL8Rh KO mice are denoted as "WT day 0" and "KO day 0", respectively. Cell counts obtained on the day following completion of aerosolized allergen challenge of WT and IL8Rh KO mice are denoted as "WT day 21" and "KO day 21", respectively.

Figure 32 is a graph depicting a secondary IgE response in WT and IL8Rh KO mice initially challenged with ovalbumin allergen ip inoculation and subsequently challenged with allergen aerosolization. 10 Allergen-specific IgE titers for WT and IL8Rh KO control mice challenged with aerosolized allergen without prior allergen challenge are denoted as "WT OVA aero" and "KO OVA aero", respectively. Allergen-specific IgE titers for WT and IL8Rh KO mice initially challenged with allergen by ip inoculation and subsequently exposed to aerosolized allergen are denoted as "WT OVA ip/aero" and "KO OVA ip/aero", respectively.

Figures 33-35 are photomicrographs depicting the lung histology of the most severely affected WT 15 asthmatic mouse. Photomicrographs of a single specimen of lung tissue were taken at 40x, 200x and 320x magnification, shown in Figures 33-35, respectively. Figure 33 shows that almost every bronchus is heavily infiltrated with granulocytes. A higher magnification of the most affected area (shown in Figure 34) reveals a strong monocytic infiltrate with several eosinophils. At the alveolar level (the highest magnification, shown in Figure 35), macrophages and eosinophils are apparent.

20 Figures 36-38 are photomicrographs depicting the lung histology of the least affected WT asthmatic mouse. Photomicrographs of a single specimen of lung tissue were taken at 40x, 200x and 320x magnification, shown in Figures 36-38, respectively. As shown in Figures 36 and 37, the least affected WT asthmatic mouse presents cell infiltrates in most bronchii, albeit less extensive than the infiltration presented by the most affected WT animal (Figures 33-35). The alveoli of the least affected WT animal are also less obstructed as shown in 25 Figure 38.

Figures 39-41 are photomicrographs depicting the lung histology of the most severely affected IL8Rh 30 KO asthmatic mouse. Photomicrographs of a single specimen of lung tissue were taken at 40x, 200x and 320x magnification, shown in Figures 39-41, respectively. Figure 39 shows that only the larger bronchii are infiltrated. The extent of infiltration shown in Figures 39 and 40 is comparable to that shown in Figures 36 and 37 for the least affected WT mouse. At the alveolar level, only a few infiltrating cells are apparent in the most severely affected IL8Rh KO specimen (Figure 41).

DESCRIPTION OF THE PREFERRED EMBODIMENTS

A. DEFINITIONS

In general, the following words or phrases have the indicated definition when used in the description, 35 examples, and claims.

"Polymerase chain reaction" or "PCR" refers to a procedure or technique in which minute amounts of a specific piece of nucleic acid, RNA and/or DNA, are amplified as described in U.S. Patent No. 4,683,195 issued 28 July 1987. Generally, sequence information from the ends of the region of interest or beyond needs

to be available, such that oligonucleotide primers can be designed; these primers will be identical or similar in sequence to opposite strands of the template to be amplified. The 5' terminal nucleotides of the two primers can coincide with the ends of the amplified material. PCR can be used to amplify specific RNA sequences, specific DNA sequences from total genomic DNA, and complementary DNA (cDNA) transcribed from total 5 cellular RNA, bacteriophage or plasmid sequences, etc. See generally Mullis et al., Cold Spring Harbor Symp. Quant. Biol. 51:263 (1987); Erlich, ed., PCR Technology (Stockton Press, NY, 1989). As used herein, PCR is considered to be one, but not the only, example of a nucleic acid polymerase reaction method for amplifying a nucleic acid test sample comprising the use of a known nucleic acid as a primer and a nucleic acid polymerase to amplify or generate a specific piece of nucleic acid.

10 "Antibodies" (Abs) and "immunoglobulins" (Igs) are glycoproteins having the same structural characteristics. While antibodies exhibit binding specificity to a specific antigen, immunoglobulins include both antibodies and other antibody-like molecules which lack antigen specificity. Polypeptides of the latter kind are, for example, produced at low levels by the lymph system and at increased levels by myelomas.

15 "Native antibodies and immunoglobulins" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies between the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other 20 end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain. Particular amino acid residues are believed to form an interface between the light- and heavy-chain variable domains (Clothia et al., J. Mol. Biol. 186:651 (1985); Novotny and Haber, Proc. Natl. Acad. Sci. U.S.A. 82:4592 (1985)).

25 The term "variable" refers to the fact that certain portions of the variable domains differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. However, the variability is not evenly distributed throughout the variable domains of antibodies. It is concentrated in three segments called complementarity-determining regions (CDRs) or hypervariable regions both in the light-chain and the heavy-chain variable domains. The more highly conserved portions of variable domains are called the framework (FR). The variable domains of native heavy 30 and light chains each comprise four FR regions, largely adopting a β -sheet configuration, connected by three CDRs, which form loops connecting, and in some cases forming part of, the β -sheet structure. The CDRs in each chain are held together in close proximity by the FR regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding site of antibodies (see Kabat et al., Sequences of Proteins of Immunological Interest, Fifth Edition, National Institute of Health, Bethesda, MD (1991)). The constant 35 domains are not involved directly in binding an antibody to an antigen, but exhibit various effector functions, such as participation of the antibody in antibody-dependent cellular toxicity.

Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site, and a residual "Fc" fragment, whose name reflects its ability

to crystallize readily. Pepsin treatment yields an $F(ab')_2$ fragment that has two antigen-combining sites and is still capable of cross-linking antigen.

"Fv" is the minimum antibody fragment which contains a complete antigen-recognition and -binding site. This region consists of a dimer of one heavy- and one light-chain variable domain in tight, non-covalent 5 association. It is in this configuration that the three CDRs of each variable domain interact to define an antigen-binding site on the surface of the V_H - V_L dimer. Collectively, the six CDRs confer antigen-binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) has the ability to recognize and bind antigen, although at a lower affinity than the entire binding site.

10 The Fab fragment also contains the constant domain of the light chain and the first constant domain (C_H1) of the heavy chain. Fab' fragments differ from Fab fragments by the addition of a few residues at the carboxy terminus of the heavy chain C_H1 domain including one or more cysteines from the antibody hinge region. Fab'-SH is the designation herein for Fab' in which the cysteine residue(s) of the constant domains bear a free thiol group. $F(ab')_2$ antibody fragments originally were produced as pairs of Fab' fragments which have 15 hinge cysteines between them. Other chemical couplings of antibody fragments are also known.

The "light chains" of antibodies (immunoglobulins) from any vertebrate species can be assigned to one of two clearly distinct types, called kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

Depending on the amino acid sequence of the constant domain of their heavy chains, immunoglobulins 20 can be assigned to different classes. There are five major classes of immunoglobulins: IgA, IgD, IgE, IgG, and IgM, and several of these can be further divided into subclasses (isotypes), e.g., IgG₁, IgG₂, IgG₃, IgG₄, IgA₁, and IgA₂. The heavy-chain constant domains that correspond to the different classes of immunoglobulins are called α , δ , ϵ , γ , and μ , respectively. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known.

25 The term "antibody" is used in the broadest sense and specifically covers single monoclonal antibodies (including agonist and antagonist antibodies) and antibody compositions with polyepitopic specificity.

The term "monoclonal antibody" (mAb) as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. 30 Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each mAb is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they can be synthesized by hybridoma culture, uncontaminated by other immunoglobulins.

35 The monoclonal antibodies herein include hybrid and recombinant antibodies produced by splicing a variable (including hypervariable) domain of an anti-IL-8 antibody with a constant domain (e.g. "humanized" antibodies), or a light chain with a heavy chain, or a chain from one species with a chain from another species, or fusions with heterologous proteins, regardless of species of origin or immunoglobulin class or subclass designation, as well as antibody fragments (e.g., Fab, $F(ab')_2$, and Fv), so long as they exhibit the desired

biological activity. (See, e.g., Cabilly et al., U.S. Pat. No. 4,816,567; Mage and Lamoyi, in Monoclonal Antibody Production Techniques and Applications, pp. 79-97 (Marcel Dekker, Inc., New York, 1987).)

Thus, the modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the 5 antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention can be made by the hybridoma method first described by Kohler and Milstein, Nature 256:495 (1975), or can be made by recombinant DNA methods (Cabilly et al., *supra*).

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences 10 in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (Cabilly et al., *supra*; Morrison et al., Proc. Natl. Acad. Sci. U.S.A. 81:6851 (1984)).

15 "Humanized" forms of non-human (e.g., murine) antibodies are specific chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂, or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from 20 a complementarity-determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat, or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies can comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. These modifications are made to further refine and optimize antibody performance. In general, the humanized antibody will comprise 25 substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details see Jones et al., Nature 321:522 (1986); Reichmann et al., Nature 332:323 (1988); and Presta, 30 Curr. Op. Struct. Biol. 2:593 (1992).

The term "IL-8 antagonist" as used herein denotes a compound capable of disrupting or blocking the interaction between IL-8 and IL-8 receptor. IL-8 antagonists include anti-IL-8 antibodies and fragments thereof, IL-8-binding peptides and nonproteinaceous small molecules capable of binding to IL-8 or competing with IL-8 for binding to IL-8 receptor.

35 "Treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Those in need of treatment include those already with the disorder as well as those prone to have the disorder or those in which the disorder is to be prevented.

"Mammal" for purposes of treatment refers to any animal classified as a mammal, including humans, domestic and farm animals, and zoo, sports, or pet animals, such as dogs, horses, cats, cows, etc. Preferably, the mammal herein is human.

As used herein, protein, peptide and polypeptide are used interchangeably to denote an amino acid 5 polymer or a set of two or more interacting or bound amino acid polymers.

As used herein, the term "inflammatory disorders" refers to pathological states resulting in 10 inflammation, typically caused by neutrophil chemotaxis. Examples of such disorders include inflammatory skin diseases including psoriasis; responses associated with inflammatory bowel disease (such as Crohn's disease and ulcerative colitis); ischemic reperfusion; adult respiratory distress syndrome; dermatitis; meningitis; 15 encephalitis; uveitis; autoimmune diseases such as rheumatoid arthritis, Sjorgen's syndrome, vasculitis; diseases involving leukocyte diapedesis; central nervous system (CNS) inflammatory disorder, multiple organ injury syndrome secondary to septicaemia or trauma; alcoholic hepatitis, bacterial pneumonia, antigen-antibody complex mediated diseases; inflammations of the lung, including pleurisy, alveolitis, vasculitis, pneumonia, chronic bronchitis, bronchiectasis, and cystic fibrosis; etc. The preferred indications are bacterial pneumonia 20 and inflammatory bowel disease such as ulcerative colitis.

As used herein, the terms "asthma", "asthmatic disorder", "asthmatic disease", and "bronchial asthma" refer to a condition of the lungs in which there is widespread narrowing of lower airways. "Atopic asthma" and "allergic asthma" refer to asthma that is a manifestation of an IgE-mediated hypersensitivity reaction in the lower airways, including, e.g., moderate or severe chronic asthma, such as conditions requiring the frequent 20 or constant use of inhaled or systemic steroids to control the asthma symptoms. A preferred indication is allergic asthma.

B. MODES FOR CARRYING OUT THE INVENTION

I. IL-8 Antagonist Preparation

The methods of the present invention can be practiced with any IL-8 antagonist that is capable of 25 inhibiting or blocking IL-8 binding to neutrophils. Preferably, the IL-8 antagonist is capable of inhibiting neutrophil chemotaxis in response to IL-8 and/or capable of inhibiting the IL-8 mediated elastase release of neutrophils. IL-8 antagonists suitable for use herein include anti-IL-8 antibodies, IL-8 binding peptides, and nonproteinaceous small molecules capable of disrupting or blocking the interaction between IL-8 and its 30 receptors. Candidate IL-8 antagonists can be tested for inhibition of IL-8 binding to neutrophils, inhibition of IL-8 mediated neutrophil chemotaxis, and inhibition of IL-8 mediated neutrophil elastase release as follows.

1. Inhibition of IL-8 binding to neutrophils

Preferably, the candidate IL-8 antagonist is tested for the ability to inhibit IL-8 binding to neutrophils of the same mammal species as that of the patient intended for IL-8 antagonist therapy. In one embodiment, neutrophils obtained from the patient are used to test candidate IL-8 antagonists, enabling the 35 physician to identify the agents with greatest therapeutic efficacy for the particular patient. However, the invention also encompasses the use of neutrophils obtained from a species of mammal other than that of the intended patient for assessing the therapeutic potential of a candidate IL-8 antagonist. Neutrophils can be

separated from red cells and mononuclear cells by sedimentation of whole blood in 1.5% Dextran T500 (Pharmacia, Sweden), layering the supernatant on a Lymphocyte Separation Medium (Organon Teknika, Durham, NC) and centrifuging according to the manufacturer's directions, recovering the cell pellet, and then subjecting the cell pellet to 2 or 3 cycles of hypotonic lysis. Alternatively, neutrophils can be separated from 5 red blood cells and peripheral blood mononuclear cells by laying whole blood samples on Mono-Poly Resolving Medium (M-PRM) (Flow Laboratories, McLean, VA) and recovering the neutrophil band according to the vendor's directions.

Similarly, it is preferable to use IL-8 from the same mammal species as that of the intended patient in testing a candidate IL-8 antagonist's ability to inhibit IL-8 binding to neutrophils. However, it is within the 10 scope of the invention to use IL-8 derived from any mammalian species provided that the IL-8 binds to the neutrophils selected for testing with the candidate IL-8 antagonist. Preferably, the IL-8 and neutrophils used for testing are derived from the same mammalian species.

IL-8 can be isolated in vitro from endothelial cells or activated T cells and monocytes derived from the species of interest. IL-8 can be conveniently harvested from endothelial cells according to the method of 15 Gimbrone *et al.*, Science, **246**: 1601 (1989) or from activated T cells and monocytes according to the method of Lindley *et al.*, Proc. Natl. Acad. Sci., **85**: 9199 (1988).

If the amino acid sequence of the particular IL-8 species is known, such as the amino acid sequence of human IL-8 (disclosed in Walz *et al.*, Biochem. Biophys. Res. Comm., **149**: 755-761 (1987); Yoshimura *et al.*, Proc. Natl. Acad. Sci. USA, **84**: 9233-9237 (1987); Van Damme *et al.*, J. Exp. Med., **167**: 1364-1376 20 (1988); Gregory *et al.*, Biochem. Biophys. Res. Comm., **151**: 883-890 (1988)), the IL-8 species of interest can be chemically synthesized, e.g., by using the solid phase synthesis method described by Merrifield, Science, **232**: 342-347 (1986). In this method, a growing polypeptide chain is covalently anchored, usually by its C-terminus, to an insoluble solid support such as beads of polystyrene resin, and the appropriately blocked amino acids and reagents are added in the proper sequence. This permits the quantitative recovery of the desired IL-8 25 product by simply filtering and washing the beads.

Alternatively, the IL-8 species of interest is produced by recombinant techniques. Recombinant IL-8 can be obtained by isolating or synthesizing DNA encoding the desired IL-8, cloning the IL-8 encoding DNA into an appropriate expression vector, transfecting a suitable expression host cell with the recombinant vector, selecting or detecting recombinant host cells, and growing the recombinant host cells under conditions 30 permitting expression of IL-8 and harvesting the IL-8 produced thereby. In a preferred embodiment, recombinant human IL-8 is obtained as described in Hebert *et al.*, J. Immunol., **145**: 3033-3040 (1990).

Commercially available IL-8 species are also suitable for use herein. For example, recombinant human IL-8 can be purchased from R&D Systems, Minneapolis, MN (catalog no. 208-IL in the 1995 Catalog).

Any method for assaying IL-8 binding to neutrophils can be used to test a candidate IL-8 antagonist. 35 Suitable assays include competitive binding assays wherein IL-8 binding to neutrophils is measured in the presence and absence of the candidate IL-8 antagonist. The IL-8 binding can be conveniently detected with the use of labelled IL-8, e.g., radiolabels, fluorochrome labels, enzyme labels, spin labels, etc., or with labelled anti-IL-8 antibodies. In a typical IL-8 competitive binding assay, the neutrophils are suspended in an appropriate buffer solution containing various concentrations of the candidate IL-8 antagonist, the labelled IL-8

is admixed to the cell suspension, the mixture is incubated under conditions allowing IL-8 to bind to neutrophils for a period of time sufficient for the competitive binding reaction to reach equilibrium, unbound labelled IL-8 is removed by centrifuging or filtering the cell suspension, and labelled IL-8 bound to neutrophils is quantitated by detection of the label, e.g., scintillation counting for radiolabels, addition of chromogenic substrate and spectrophotometric assay for chromogenic enzyme labels, flow-activated cell sorting for fluorochrome labels, etc.

5 In a preferred embodiment, the candidate IL-8 antagonist is screened for inhibition of human IL-8 binding to human neutrophils as described in the Examples below.

10 The percentage of IL-8 binding inhibition at a particular concentration of candidate IL-8 antagonist can be calculated with the quotient formed by division of the amount of labelled IL-8 specifically binding to neutrophils in the presence of the agent with the total amount of labelled IL-8 specifically binding to neutrophils in the absence of the agent. Labelled IL-8 specific binding amounts can be determined by subtracting the amount of labelled non-specific binding from the total amount of labelled IL-8 binding. The amount of labelled IL-8 non-specific binding can be determined by measuring labelled IL-8 binding in the 15 presence of an excess of unlabelled IL-8. The concentration of candidate IL-8 antagonist necessary for 50% inhibition of IL-8 binding (IC_{50}) is determined using the inhibition percentages for the various concentrations of agent tested.

20 An agent is scored as positive for inhibition of IL-8 binding to neutrophils if a concentration of the agent of about 100 nanomoles/liter (nM) or lower, and preferably 1 nM or lower, and more preferably 10 picomoles/liter (pM) or lower, in the presence of an IL-8 concentration of about 0.5 nM produces decreased IL-8 binding to neutrophils in comparison to a control sample containing the same IL-8 concentration in the absence of the agent. Preferably, the candidate IL-8 antagonist is capable of inhibiting human IL-8 binding to human neutrophils with an IC_{50} of about 50 nM or less, and preferably an average IC_{50} of about 7.5 nM or less, and more preferably an average IC_{50} of about 1.6 nM or less, in the presence of human IL-8 at a 25 concentration of about 0.5 nM.

2. Inhibition of IL-8 mediated neutrophil chemotaxis

30 Preferably, the candidate IL-8 antagonist is also tested for the ability to inhibit neutrophil chemotaxis in response to IL-8. It is desirable to test the candidate IL-8 antagonist for the ability to inhibit IL-8 mediated chemotaxis of neutrophils derived from the same mammal species as that of the patient intended for IL-8 antagonist therapy. In one embodiment, neutrophils obtained from the patient are used to test candidate IL-8 antagonists, enabling the physician to identify the agents with greatest therapeutic efficacy for the particular patient. However, the invention also encompasses the use of neutrophils obtained from a species of mammal other than that of the intended patient for assessing the therapeutic potential of a candidate IL-8 antagonist.

35 Similarly, it is preferable to use IL-8 from the same mammal species as that of the intended patient in testing a candidate IL-8 antagonist's ability to inhibit IL-8 mediated neutrophil chemotaxis. However, it is within the scope of the invention to use IL-8 derived from any mammalian species provided that the IL-8 elicits

chemotaxis of the neutrophils selected for testing with the candidate IL-8 antagonist. Preferably, the IL-8 and neutrophils used for testing are derived from the same mammalian species.

In one aspect, a candidate IL-8 antagonist is tested for inhibition of IL-8 mediated neutrophil chemotaxis using a 96 well microtiter chemotaxis apparatus (Neuro Probe, Cabin John, Maryland) wherein 5 each well is horizontally divided into two chambers by a 5 micron filter. A sample of the desired IL-8 is obtained as described in Section I above, combined with a particular concentration of the candidate IL-8 antagonist, and then placed in the bottom chamber of the chemotaxis apparatus. A sample of the desired neutrophils is obtained as described in Section I above and the cells are labelled with the fluorescent dye 10 calcein AM (Molecular Probe, Eugene, OR). The cells are washed, resuspended in an appropriate buffer, counted and placed in the top chamber of the chemotaxis apparatus. The chemotaxis apparatus is incubated under conditions permitting IL-8 to diffuse into the neutrophil loading (top) chamber for a period of time sufficient to elicit neutrophil migration into the adjoining chamber. After incubation, cells remaining in the neutrophil loading (top) chamber are removed by aspiration and the top chamber side of the filter is washed and scraped to remove non-migrating cells. Labelled neutrophils in the bottom chamber and on the bottom 15 chamber side of the filter are then quantitated for analysis.

In a preferred embodiment, the candidate IL-8 antagonist is assayed for inhibition of human neutrophil chemotaxis in response to human IL-8 as described in the Examples below.

The relative number of migrating and non-migrating neutrophils in a sample containing IL-8 antagonist can be determined by comparison of the signal detected in the IL-8 antagonist sample with the signal 20 detected in a sample containing IL-8 alone (providing the positive control for uninhibited IL-8 induced migration) and the signal detected in a sample containing buffer alone (providing the negative control for background migration). An agent is scored as positive for inhibition of IL-8 mediated neutrophil migration if a concentration of the agent of about 100 nM or lower, and preferably 1 nM or lower, and more preferably 10 pM or lower, in the presence of an initial IL-8 concentration of about 2 nM produces decreased neutrophil 25 migration in comparison to a control sample containing the same IL-8 concentration in the absence of the agent. Preferably, the candidate IL-8 antagonist inhibits 50% of human neutrophil migration at a concentration of about 6.0 nM or less, and more preferably at a concentration of about 3.0 nM or less, in the presence of an initial human IL-8 concentration of about 4 nM.

3. Inhibition of IL-8 mediated neutrophil elastase release

30 Preferably, the candidate IL-8 antagonist is further tested for the ability to inhibit neutrophil elastase release in response to IL-8. It is desirable to test the candidate IL-8 antagonist for the ability to inhibit IL-8 mediated elastase release of neutrophils derived from the same mammal species as that of the patient intended for IL-8 antagonist therapy. In one embodiment, neutrophils obtained from the patient are used to test candidate IL-8 antagonists, enabling the physician to identify the agents with greatest therapeutic efficacy 35 for the particular patient. However, the invention also encompasses the use of neutrophils obtained from a species of mammal other than that of the intended patient for assessing the therapeutic potential of a candidate IL-8 antagonist.

Similarly, it is preferable to use IL-8 from the same mammal species as that of the intended patient in testing a candidate IL-8 antagonist's ability to inhibit IL-8 mediated neutrophil release of elastase. However, it is within the scope of the invention to use IL-8 derived from any mammalian species provided that the IL-8 induces elastase release in the neutrophils selected for testing with the candidate IL-8 antagonist. Preferably, 5 the IL-8 and neutrophils used for testing are derived from the same mammalian species.

In non-stimulated neutrophils, IL-8 does not trigger the release of azurophil granules. In the presence of cytochalasin B, IL-8 causes degranulation of the azurophil granules and release of elastase. Thus, the ability of a candidate IL-8 antagonist to inhibit neutrophil elastase release in response to IL-8 can be determined by 10 obtaining the desired neutrophils and IL-8 as described in Section I above, incubating the neutrophils in suspension with cytochalasin B, incubating the cytochalasin B-primed neutrophils with IL-8 in the presence or absence of the candidate IL-8 antagonist, centrifuging the cell suspension to remove the cells, incubating the cell-free supernatants with the elastase substrate methoxysuccinyl-alanyl-alanyl-prolyl-valyl-p-nitroanilide, and detecting the presence of p-nitroaniline in the test samples by spectrophotometric analysis at a wavelength 15 of 405 nanometers (nm).

15 In a preferred embodiment, the candidate IL-8 antagonist is assayed for inhibition of human neutrophil elastase release in response to human IL-8 as described in the Examples below.

The inhibition percentage of IL-8 mediated neutrophil elastase release at a particular concentration 20 of candidate IL-8 antagonist can be calculated with the quotient formed by dividing the 405 nm fluorescence detected in the candidate IL-8 antagonist treated sample's supernatant by the 405 nm fluorescence detected in the IL-8 treated control sample's supernatant. An agent is scored as positive for inhibition of IL-8 mediated neutrophil elastase release if a concentration of the agent of about 10 micromoles/liter (μ M) or lower, and preferably 100 nM or lower, and more preferably 1 nM or lower, in the presence of an IL-8 concentration of about 100 nM produces decreased neutrophil elastase release in comparison to a control sample for the same 25 IL-8 concentration in the absence of the agent. Preferably, the candidate IL-8 antagonist inhibits 50% of human neutrophil elastase release induced by human IL-8 at a candidate IL-8 antagonist:human IL-8 molar ratio of about 1.0 or less, and more preferably about 0.65 or less.

II. Anti-IL-8 antibody preparation

1. Monoclonal antibodies

The anti-IL-8 antibodies of the invention are preferably monoclonal, binding IL-8 with a 30 dissociation constant (K_d) of about 1×10^{-8} to 1×10^{-11} , more preferably, 1×10^{-9} to 1×10^{-10} . The antibodies of the invention preferably do not measurably bind in an enzyme-linked immunoabsorbent assay (ELISA) to chemokines other than IL-8, such as C5a, platelet factor 4 or β -TG. Furthermore, the antibodies of the invention preferably inhibit elastase release from IL-8 stimulated neutrophils and inhibit IL-8 stimulated chemotaxis of neutrophils. In one embodiment of the invention, the antibodies of the invention can bind IL-8 35 from non-human species in addition to human IL-8, such as rabbit IL-8.

In another embodiment of the invention, Fab, Fab', Fab'-SH, or $F(ab')_2$ fragments of the anti-IL-8 antibodies of the instant invention are created. These antibody "fragments" can be created by traditional means, such as enzymatic digestion, or may be generated by recombinant techniques. Such antibody fragments

may be chimeric or humanized. These fragments are useful for the diagnostic and therapeutic purposes set forth below.

The anti-IL-8 monoclonal antibodies of the invention can be made, for example, using the hybridoma method first described by Kohler and Milstein, Nature 256:495 (1975), or can be made by recombinant DNA methods (Cabilly et al., *supra*).

In the hybridoma method, a mouse or other appropriate host animal, such as a hamster, is immunized as hereinabove described to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the IL-8 or IL-8 fragment used for immunization. Antibodies to IL-8 generally are raised in animals by multiple subcutaneous (sc) or intraperitoneal (ip) injections of the IL-8 and an adjuvant. Animals 10 ordinarily are immunized against immunogenic conjugates or derivatives of IL-8 with monophosphoryl lipid A (MPL)/trehalose dicorynomycolate (TDM) (Ribi Immunochem. Research, Inc., Hamilton, MT) and the solution is injected intradermally at multiple sites. Two weeks later the animals are boosted. 7 to 14 days later animals are bled and the serum is assayed for anti-IL-8 titer. Animals are boosted until the titer plateaus.

Alternatively, lymphocytes can be immunized in vitro. Lymphocytes then are fused with myeloma 15 cells using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, Monoclonal Antibodies: Principles and Practice pp. 59-103 (Academic Press, 1986)).

The hybridoma cells thus prepared are seeded and grown in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, parental myeloma cells. For example, if the parental myeloma cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase 20 (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine (HAT medium), which substances prevent the growth of HGPRT-deficient cells.

Preferred myeloma cells are those that fuse efficiently, support stable high-level production of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. Among these, preferred myeloma cell lines are murine myeloma lines, such as those derived from MOPC-21 25 and MPC-11 mouse tumors available from the Salk Institute Cell Distribution Center, San Diego, California U.S.A., and SP-2 cells available from the American Type Culture Collection, Rockville, Maryland U.S.A.

Culture medium in which hybridoma cells are growing is assayed for production of monoclonal 30 antibodies directed against IL-8. Preferably, the binding specificity of monoclonal antibodies produced by hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunoabsorbent assay (ELISA).

The binding affinity of the mAbs can, for example, be determined by the Scatchard analysis of Munson and Pollard, Anal. Biochem. 107:220 (1980).

After hybridoma cells are identified that produce antibodies of the desired specificity, affinity, and/or 35 activity, the clones can be subcloned by limiting dilution procedures and grown by standard methods (Goding, *supra*). Suitable culture media for this purpose include, for example, D-MEM or RPMI-1640 medium. In addition, the hybridoma cells can be grown *in vivo* as ascites tumors in an animal.

The monoclonal antibodies secreted by the subclones are suitably separated from the culture medium, ascites fluid, or serum by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

DNA encoding the monoclonal antibodies of the invention is readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of such DNA. Once isolated, the DNA can be placed into expression vectors, which are then 5 transfected into host cells such as *E. coli* cells, simian COS cells, Chinese hamster ovary (CHO) cells, or myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. Review articles on recombinant expression in bacteria of DNA encoding the antibody include Skerra et al., *Curr. Opinion in Immunol.* 5:256 (1993) and Plückthun *Immunol. Revs.* 130:151 (1992).

10 The DNA also can be modified, for example, by substituting the coding sequence for human heavy- and light-chain constant domains in place of the homologous murine sequences (e.g., Morrison et al., *Proc. Natl. Acad. Sci.* 81:6851 (1984)), or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. In that manner, "chimeric" or "hybrid" antibodies are prepared that have the binding specificity of an anti-IL-8 mAb herein.

15 Typically, such non-immunoglobulin polypeptides are substituted for the constant domains of an antibody of the invention, or they are substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody comprising one antigen-combining site having specificity for a IL-8 and another antigen-combining site having specificity for a different antigen.

20 Chimeric or hybrid antibodies also can be prepared in vitro using known methods in synthetic protein chemistry, including those involving crosslinking agents. For example, immunotoxins can be constructed using a disulfide-exchange reaction or by forming a thioether bond. Examples of suitable reagents for this purpose include iminothiolate and methyl-4-mercaptopbutyrimidate.

2. Humanized antibodies

25 Methods for humanizing non-human antibodies are well known in the art. Generally, a humanized antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as "import" residues, which are typically taken from an "import" variable domain. Humanization can be essentially performed following the method of Winter and co-workers (Jones et al., *Nature* 321:522 (1986); Riechmann et al., *Nature* 332:323 (1988); Verhoeyen et al., *Science* 239:1534 (1988)), by substituting rodent CDRs or CDR sequences for the corresponding sequences 30 of a human antibody. Accordingly, such "humanized" antibodies are chimeric antibodies (Cabilly et al., *supra*), wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically human antibodies in which some CDR residues and possibly some FR residues are substituted by residues from analogous sites in rodent antibodies.

35 The choice of human variable domains, both light and heavy, to be used in making the humanized antibodies is very important to reduce antigenicity. According to the so-called "best-fit" method, the sequence of the variable domain of a rodent antibody is screened against the entire library of known human variable-domain sequences. The human sequence which is closest to that of the rodent is then accepted as the human

framework (FR) for the humanized antibody (Sims et al., J. Immunol. 151: 2296 (1993); Chothia and Lesk, J. Mol. Biol. 196:901 (1987)). Another method uses a particular framework derived from the consensus sequence of all human antibodies of a particular subgroup of light or heavy chains. The same framework can be used for several different humanized antibodies (Carter et al., Proc. Natl. Acad. Sci. U.S.A. 89:4285 (1992); 5 Presta et al., J. Immunol. 151:2623 (1993)).

It is further important that antibodies be humanized with retention of high affinity for the antigen and other favorable biological properties. To achieve this goal, according to a preferred method, humanized antibodies are prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional 10 immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be 15 selected and combined from the consensus and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is achieved. In general, the CDR residues are directly and most substantially involved in influencing antigen binding.

3. Human antibodies

Human monoclonal antibodies can be made by the hybridoma method. Human myeloma and 20 mouse-human heteromyeloma cell lines for the production of human monoclonal antibodies have been described, for example, by Kozbor, J. Immunol. 133:3001 (1984); Brodeur et al., Monoclonal Antibody Production Techniques and Applications, pp. 51-63 (Marcel Dekker, Inc., New York, 1987); and Boerner et al., J. Immunol. 147:86 (1991).

It is now possible to produce transgenic animals (e.g., mice) that are capable, upon immunization, of 25 producing a full repertoire of human antibodies in the absence of endogenous immunoglobulin production. For example, it has been described that the homozygous deletion of the antibody heavy-chain joining region (J_H) gene in chimeric and germ-line mutant mice results in complete inhibition of endogenous antibody production. Transfer of the human germ-line immunoglobulin gene array in such germ-line mutant mice will result in the production of human antibodies upon antigen challenge. See, e.g., Jakobovits et al., Proc. Natl. 30 Acad. Sci. U.S.A. 90:2551 (1993); Jakobovits et al. Nature 362:255 (1993); Bruggermann et al., Year in Immuno. 7:33 (1993).

Alternatively, phage display technology (McCafferty et al., Nature 348:552 (1990)) can be used to produce human antibodies and antibody fragments in vitro, from immunoglobulin variable (V) domain gene 35 repertoires from unimmunized donors. According to this technique, antibody V domain genes are cloned in-frame into either a major or minor coat protein gene of a filamentous bacteriophage, such as M13 or fd, and displayed as functional antibody fragments on the surface of the phage particle. Because the filamentous particle contains a single-stranded DNA copy of the phage genome, selections based on the functional properties of the antibody also result in selection of the gene encoding the antibody exhibiting those properties.

Thus, the phage mimics some of the properties of the B-cell. Phage display can be performed in a variety of formats; for their review see, e.g., Johnson et al., Current Opinion in Structural Biology 3:564 (1993). Several sources of V-gene segments can be used for phage display. Clackson et al., Nature 352:624 (1991) isolated a diverse array of anti-oxazolone antibodies from a small random combinatorial library of V genes derived 5 from the spleens of immunized mice. A repertoire of V genes from unimmunized human donors can be constructed and antibodies to a diverse array of antigens (including self-antigens) can be isolated essentially following the techniques described by Marks et al., J. Mol. Biol. 222:581 (1991), or Griffith et al., EMBO J. 12:725 (1993).

In a natural immune response, antibody genes accumulate mutations at a high rate (somatic 10 hypermutation). Some of the changes introduced will confer higher affinity, and B cells displaying high-affinity surface immunoglobulin are preferentially replicated and differentiated during subsequent antigen challenge. This natural process can be mimicked by employing the technique known as "chain shuffling" (Marks et al., Bio/Technol. 10:779 (1992)). In this method, the affinity of "primary" human antibodies obtained by phage display can be improved by sequentially replacing the heavy and light chain V region genes 15 with repertoires of naturally occurring variants (repertoires) of V domain genes obtained from unimmunized donors. This technique allows the production of antibodies and antibody fragments with affinities in the nM range. A strategy for making very large phage antibody repertoires has been described by Waterhouse et al., Nucl. Acids Res. 21:2265 (1993).

Gene shuffling can also be used to derive human antibodies from rodent antibodies, where the human 20 antibody has similar affinities and specificities to the starting rodent antibody. According to this method, which is also referred to as "epitope imprinting", the heavy or light chain V domain gene of rodent antibodies obtained by phage display technique is replaced with a repertoire of human V domain genes, creating rodent-human chimeras. Selection with antigen results in isolation of human variable capable of restoring a functional 25 antigen-binding site, i.e. the epitope governs (imprints) the choice of partner. When the process is repeated in order to replace the remaining rodent V domain, a human antibody is obtained (see PCT WO 93/06213, published 1 April 1993). Unlike traditional humanization of rodent antibodies by CDR grafting, this technique provides completely human antibodies, which have no framework or CDR residues of rodent origin.

4. Bispecific antibodies

Bispecific antibodies are monoclonal, preferably human or humanized, antibodies that have 30 binding specificities for at least two different antigens. In the present case, one of the binding specificities is for IL-8, the other one is for any other antigen. For example, bispecific antibodies specifically binding a IL-8 and neurotrophic factor, or two different types of IL-8 polypeptides are within the scope of the present invention.

Methods for making bispecific antibodies are known in the art. Traditionally, the recombinant 35 production of bispecific antibodies is based on the co-expression of two immunoglobulin heavy chain-light chain pairs, where the two heavy chains have different specificities (Milstein and Cuello, Nature 305:537 (1983)). Because of the random assortment of immunoglobulin heavy and light chains, these hybridomas (quadromas) produce a potential mixture of 10 different antibody molecules, of which only one has the correct

bispecific structure. The purification of the correct molecule, which is usually done by affinity chromatography steps, is rather cumbersome, and the product yields are low. Similar procedures are disclosed in WO 93/08829 published 13 May 1993, and in Traunecker et al., EMBO J. 10:3655 (1991).

According to a different and more preferred approach, antibody-variable domains with the desired binding specificities (antibody-antigen combining sites) are fused to immunoglobulin constant-domain sequences. The fusion preferably is with an immunoglobulin heavy-chain constant domain, comprising at least part of the hinge, the second heavy chain constant region ($C_{H}2$), and the third heavy chain constant region ($C_{H}3$). It is preferred to have the first heavy-chain constant region ($C_{H}1$), containing the site necessary for light-chain binding, present in at least one of the fusions. DNAs encoding the immunoglobulin heavy chain fusions and, if desired, the immunoglobulin light chain, are inserted into separate expression vectors, and are co-transfected into a suitable host organism. This provides for great flexibility in adjusting the mutual proportions of the three polypeptide fragments in embodiments when unequal ratios of the three polypeptide chains used in the construction provide the optimum yields. It is, however, possible to insert the coding sequences for two or all three polypeptide chains in one expression vector when the production of at least two polypeptide chains in equal ratios results in high yields or when the ratios are of no particular significance. In a preferred embodiment of this approach, the bispecific antibodies are composed of a hybrid immunoglobulin heavy chain with a first binding specificity in one arm, and a hybrid immunoglobulin heavy chain-light chain pair (providing a second binding specificity) in the other arm. This asymmetric structure facilitates the separation of the desired bispecific compound from unwanted immunoglobulin chain combinations, as the presence of an immunoglobulin light chain in only one half of the bispecific molecule provides for a facile way of separation. For further details of generating bispecific antibodies, see, for example, Suresh et al., Methods in Enzymology 121:210 (1986).

5. Heteroconjugate antibodies

Heteroconjugate antibodies are also within the scope of the present invention. Heteroconjugate antibodies are composed of two covalently joined antibodies. Such antibodies have, for example, been proposed to target immune system cells to unwanted cells (U.S. Patent No. 4,676,980), and for treatment of HIV infection (WO 91/00360; WO 92/00373; and EP 03089). Heteroconjugate antibodies can be made using any convenient cross-linking methods. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

30 III. Diagnostic uses of anti-IL-8 antibodies

For diagnostic applications requiring the detection or quantitation of IL-8, the antibodies of the invention typically will be labeled with a detectable moiety. The detectable moiety can be any one which is capable of producing, either directly or indirectly, a detectable signal. For example, the detectable moiety can be a radioisotope, such as ^{3}H , ^{14}C , ^{32}P , ^{35}S , or ^{125}I ; a fluorescent or chemiluminescent compound, such as fluorescein isothiocyanate, rhodamine, or luciferin; radioactive isotopic labels, such as, e.g., ^{125}I , ^{32}P , ^{14}C , or ^{3}H ; or an enzyme, such as alkaline phosphatase, beta-galactosidase, or horseradish peroxidase.

Any method known in the art for separately conjugating the antibody to the detectable moiety can be employed, including those methods described by Hunter et al., Nature 144:945 (1962); David et al., Biochemistry 13:1014 (1974); Pain et al., J. Immunol. Meth. 40:219 (1981); and Nygren, J. Histochem. and Cytochem. 30:407 (1982).

5 The antibodies of the present invention can be employed in any known assay method, such as competitive binding assays, direct and indirect sandwich assays, and immunoprecipitation assays. For example, see Zola, Monoclonal Antibodies: A Manual of Techniques, pp. 147-158 (CRC Press, Inc., 1987).

Competitive binding assays rely on the ability of a labeled standard (which can be a IL-8 or an immunologically reactive portion thereof) to compete with the test sample analyte (IL-8) for binding with a 10 limited amount of antibody. The amount of IL-8 in the test sample is inversely proportional to the amount of standard that becomes bound to the antibodies. To facilitate determining the amount of standard that becomes bound, the antibodies generally are insolubilized before or after the competition, so that the standard and analyte that are bound to the antibodies can conveniently be separated from the standard and analyte which remain unbound.

15 Sandwich assays involve the use of two antibodies, each capable of binding to a different antigenic portion, or epitope, of the protein (IL-8) to be detected. In a sandwich assay, the test sample analyte is bound by a first antibody which is immobilized on a solid support, and thereafter a second antibody binds to the analyte, thus forming an insoluble three-part complex (U.S. Patent No. 4,376,110). The second antibody can itself be labeled with a detectable moiety (direct sandwich assays) or can be measured using an anti- 20 immunoglobulin antibody that is labeled with a detectable moiety (indirect sandwich assay). For example, one type of sandwich assay is an ELISA assay, in which case the detectable moiety is an enzyme (e.g., horseradish peroxidase).

IL-8 antibodies also are useful for the affinity purification of IL-8 from recombinant cell culture or natural sources. For example, these antibodies can be fixed to a solid support by techniques well known in the 25 art so as to purify IL-8 from a source such as culture supernatant or tissue.

IV. Therapeutic compositions and administration of IL-8 antagonist

Therapeutic formulations of IL-8 antagonist are prepared for storage by IL-8 antagonist having the desired degree of purity with optional physiologically acceptable carriers, excipients, or stabilizers (Remington's Pharmaceutical Sciences, *supra*), in the form of lyophilized cake or aqueous solutions. 30 Acceptable carriers, excipients or stabilizers are nontoxic to recipients at the dosages and concentrations employed, and include buffers such as phosphate, citrate, and other organic acids; antioxidants including ascorbic acid; low molecular weight (less than about 10 residues) polypeptides; proteins, such as serum albumin, gelatin, or immunoglobulins; hydrophilic polymers such as polyvinylpyrrolidone; amino acids such as glycine, glutamine, asparagine, arginine or lysine; monosaccharides, disaccharides, and other carbohydrates 35 including glucose, mannose, or dextrans; chelating agents such as EDTA; sugar alcohols such as mannitol or sorbitol; salt-forming counterions such as sodium; and/or nonionic surfactants such as Tween, Pluronics or polyethylene glycol (PEG).

The IL-8 antagonist to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes, prior to or following lyophilization and reconstitution. The IL-8 antagonist ordinarily will be stored in lyophilized form or in solution.

Therapeutic IL-8 antagonist compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

The route of IL-8 antagonist administration is in accord with known methods, e.g., inhalation, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, or intralesional routes, by enema or suppository, or by sustained release systems.

10 In one embodiment, the invention provides for the treatment of asthmatic diseases by administration of IL-8 antagonist to the respiratory tract. The invention contemplates formulations comprising an IL-8 antagonist for use in a wide variety of devices that are designed for the delivery of pharmaceutical compositions and therapeutic formulations to the respiratory tract. In one aspect of the present invention, an IL-8 antagonist is administered in aerosolized or inhaled form. The IL-8 antagonist, combined with a dispersing agent, or
15 dispersant, can be administered in an aerosol formulation as a dry powder or in a solution or suspension with a diluent.

20 Suitable dispersing agents are well known in the art, and include but are not limited to surfactants and the like. Surfactants are generally used in the art to reduce surface induced aggregation of protein caused by atomization of the solution forming the liquid aerosol. Examples of such surfactants include polyoxyethylene fatty acid esters and alcohols, and polyoxyethylene sorbitan fatty acid esters. Amounts of surfactants used will vary, being generally within the range of about 0.001 to 4% by weight of the formulation. In a specific aspect, the surfactant is polyoxyethylene sorbitan monooleate or sorbitan trioleate.

25 The liquid aerosol formulations contain the IL-8 antagonist and a dispersing agent in a physiologically acceptable diluent. The dry powder aerosol formulations of the present invention consist of a finely divided solid form of the IL-8 antagonist and a dispersing agent, and optionally a bulking agent, such as lactose, sorbitol, sucrose, or mannitol, and the like, to facilitate dispersal of the powder. With either the liquid or dry powder aerosol formulation, the formulation must be aerosolized. That is, it must be broken down into liquid or solid particles in order to ensure that the aerosolized dose actually reaches the bronchii and/or alveoli, as desired. For example, in the methods for treatment of asthma provided herein, it is preferable to deliver 30 aerosolized IL-8 antagonist to the bronchii. In other embodiments, such as the present methods for treating adult respiratory distress syndrome, it is preferably to deliver aerosolized IL-8 antagonist to the alveoli. In general the mass median dynamic diameter will be 5 micrometers (μm) or less in order to ensure that the drug particles reach the lung bronchii or alveoli (Wearley, L.L., 1991, 1991, Crit. Rev. in Ther. Drug Carrier Systems 8:333).

35 With regard to construction of the delivery device, any form of aerosolization known in the art, including but not limited to nebulization, atomization or pump aerosolization of a liquid formulation, and aerosolization of a dry powder formulation, can be used in the practice of the invention. A delivery device that is uniquely designed for administration of solid formulations is envisioned. Often, the aerosolization of a liquid or a dry powder formulation will require a propellant. The propellant can be any propellant generally used in

the art. Examples of useful propellants include chlorofluorocarbons, hydrofluorocarbons, hydrochlorofluorocarbons, and hydrocarbons, including trifluoromethane, dichlorodifluoromethane, dichlorotetrafluoroethanol, and 1,1,1,2-tetrafluoroethane, and combinations thereof.

In a preferred aspect of the invention, the device for aerosolization is a metered dose inhaler. A 5 metered dose inhaler provides a specific dosage when administered, rather than a variable dose depending on administration. Such a metered dose inhaler can be used with either a liquid or a dry powder aerosol formulation.

Systems of aerosol delivery, such as the pressurized metered dose inhaler and the dry powder inhaler are disclosed in Newman, S.P., *Aerosols and the Lung*, Clarke, S.W. and Davia, D. editors, pp. 197-22 and can 10 be used in connection with the present invention.

Sustained release systems can be used in the practice of the methods of the invention. Suitable examples of sustained-release preparations include semipermeable polymer matrices in the form of shaped articles, e.g. films, or microcapsules. Sustained release matrices include polyesters, hydrogels, polylactides (U.S. 3,773,919, EP 58,481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 15 Biopolymers 22:547 (1983)), poly (2-hydroxyethyl-methacrylate) (Langer et al., J. Biomed. Mater. Res. 15:167 (1981) and Langer, Chem. Tech. 12:98 (1982)), ethylene vinyl acetate (Langer et al., *supra*) or poly-D-(-)-3-hydroxybutyric acid (EP 133,988). Sustained-release IL-8 antagonist compositions also include liposomally entrapped IL-8 antagonist. Liposomes containing IL-8 antagonist are prepared by methods known per se: DE 3,218,121; Epstein et al., Proc. Natl. Acad. Sci. U.S.A. 82:3688 (1985); Hwang et al., Proc. Natl. Acad. Sci. 20 U.S.A. 77:4030 (1980); EP 52,322; EP 36,676; EP 88,046; EP 143,949; EP 142,641; Japanese patent application 83-118008; U.S. Patent Nos. 4,485,045 and 4,544,545; and EP 102,324. Ordinarily the liposomes are of the small (about 200-800 Angstroms) unilamellar type in which the lipid content is greater than about 30 mole percent cholesterol, the selected proportion being adjusted for the optimal IL-8 antagonist therapy.

An "effective amount" of IL-8 antagonist to be employed therapeutically will depend, for example, 25 upon the therapeutic objectives, the route of administration, and the condition of the patient. Accordingly, it will be necessary for the therapist to titer the dosage and modify the route of administration as required to obtain the optimal therapeutic effect. Typically, the clinician will administer the IL-8 antagonist until a dosage is reached that achieves the desired effect. The progress of this therapy is easily monitored by conventional assays.

30 In the treatment and prevention of an inflammatory disorder or asthmatic disorder with an IL-8 antagonist, the IL-8 antagonist composition will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disorder being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the IL-8 antagonist, the particular type of IL-8 antagonist, the method of 35 administration, the scheduling of administration, and other factors known to medical practitioners. The "therapeutically effective amount" of antibody to be administered will be governed by such considerations, and is the minimum amount necessary to prevent, ameliorate, or treat the disorder, including treating acute or chronic respiratory diseases and reducing inflammatory responses. Such amount is preferably below the amount that is toxic to the host or renders the host significantly more susceptible to infections.

As a general proposition, the initial pharmaceutically effective amount of the IL-8 antagonist administered parenterally per dose will be in the range of about 0.1 to 50 milligrams per kilogram of patient body weight per day (mg/kg/day), with the typical initial range of IL-8 antagonist used being 0.3 to 20 mg/kg/day, more preferably 0.3 to 15 mg/kg/day.

5 In one embodiment using systemic administration, the initial pharmaceutically effective amount will be in the range of about 2 to 5 mg/kg/day.

For methods of the invention using administration by inhalation, the initial pharmaceutically effective amount will be in the range of about 1 microgram (μ g)/kg/day to 100 mg/kg/day for an antibody agent, and about 1 μ g/kg/day to 20 mg/kg/day for a small molecule agent.

10 The invention provides for both prophylactic and therapeutic treatment of asthma with IL-8 antagonists. In the case of prophylactic treatment for allergic asthma with an anti-IL-8 antibody, it is desirable to administer about 0.1 to 10 mg/kg of the antibody agent to the patient up to about 24 hours prior to anticipated exposure to allergen or prior to the onset of allergic asthma. In the case of therapeutic treatment for acute asthma, including allergic asthma, it is desirable to treat the asthmatic patient as early as possible
15 following onset of an asthma attack. In one embodiment, an episode of acute asthma is treated within 24 hours of the onset of symptoms by administration of about 0.1 to 10 mg/kg of an anti-IL-8 antibody agent. However, it will be appreciated that the methods of the invention can be used to ameliorate symptoms at any point in the pathogenesis of asthmatic disease. Additionally, the methods of the invention can be used to alleviate symptoms of chronic asthmatic conditions.

20 As noted above, however, these suggested amounts of IL-8 antagonist are subject to a great deal of therapeutic discretion. The key factor in selecting an appropriate dose and scheduling is the result obtained, as indicated above.

25 The IL-8 antagonist need not be, but is optionally formulated with one or more agents currently used to prevent or treat the inflammatory disorder or asthmatic disease in question. For example, in rheumatoid arthritis, the antibody can be given in conjunction with a glucocorticosteroid. In the case of treating asthmatic diseases with IL-8 antagonists, the invention contemplates the coadministration of IL-8 antagonist and one or more additional agents useful in treating asthma, such as bronchodilators, antihistamines, epinephrine, and the like. The effective amount of such other agents depends on the amount of IL-8 antagonist present in the formulation, the type of disorder or treatment, and other factors discussed above. These are generally used in
30 the same dosages and with administration routes as used hereinbefore or about from 1 to 99% of the heretofore employed dosages.

The following examples are offered by way of illustration and not by way of limitation. The disclosures of all citations in the specification are expressly incorporated herein by reference.

EXAMPLES

35 A. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST HUMAN IL-8

Balb/c mice were immunized in each hind footpad or intraperitoneally with 10 micrograms (μ g) of recombinant human IL-8 (produced as a fusion of (ser-IL-8)₇₂ with ubiquitin (Hebert et al. J. Immunology

145:3033-3040 (1990)); IL-8 is available commercially from PeproTech, Inc., Rocky Hill, NJ) resuspended in MPL/TDM (Ribi Immunochem. Research Inc., Hamilton, MT) and boosted twice with the same amount of IL-8. In these experiments, "IL-8" is intended to mean (ser-IL-8)₇₂ unless otherwise specified. A final boost of 10 µg of IL-8 was given 3 days before the fusion. Spleen cells or popliteal lymph node cells were fused with 5 mouse myeloma P3X63Ag8U.1 (ATCC CRL1597), a non-secreting clone of the myeloma P3X63Ag8, using 35% polyethylene glycol as described before. Ten days after the fusion, culture supernatant was screened for the presence of monoclonal antibodies to IL-8 by ELISA.

The ELISA was performed as follows. Nunc 96-well immunoplates (Flow Lab, McLean, VA) were coated with 50 microliters (µl)/well of 2 micrograms/milliliter (µg/ml) IL-8 in phosphate-buffered saline (PBS) 10 overnight at 4°C. The remaining steps were carried out at room temperature. Nonspecific binding sites were blocked with 0.5% bovine serum albumin (BSA) for 1 hour (hr). Plates were then incubated with 50 µl/well of hybridoma culture supernatants from 672 growing parental fusion wells for 1 hr, followed by the incubation with 50 µl/well of 1:1000 dilution of a 1 milligram/milliliter (mg/ml) stock solution of alkaline phosphatase-conjugated goat anti-mouse Ig (Tago Co., Foster City, CA) for 1 hr. The level of enzyme-linked antibody 15 bound to the plate was determined by the addition of 100 µl/well of 0.5 mg/ml of r-nitrophenyl phosphate in sodium bicarbonate buffer, pH 9.6. The color reaction was measured at 405 nm with an ELISA plate reader (Titertrek Multiscan, Flow Lab, McLean, VA). Between each step, plates were washed three times in PBS containing 0.05% Tween 20.

Culture supernatants which promoted 4-fold more binding of IL-8 than did control medium were 20 selected as positives. According to this criterion, 16 of 672 growing parental fusion wells (2%) were positive. These positive hybridoma cell lines were cloned at least twice by using the limiting dilution technique.

Seven of the positive hybridomas were further characterized as follows. The isotypes of the 25 monoclonal antibodies were determined by coating Nunc 96-well immunoplates (Flow Lab, McLean, VA) with IL-8 overnight, blocking with BSA, incubating with culture supernatants followed by the addition of predetermined amount of isotype-specific alkaline phosphatase-conjugated goat anti-mouse Ig (Fisher Biotech, Pittsburgh, PA). The level of conjugated antibodies bound to the plate was determined by the addition of r-nitrophenyl phosphate as described above.

All the monoclonal antibodies tested belonged to either IgG₁ or IgG₂ immunoglobulin isotype. 30 Ascites fluid containing these monoclonal antibodies had antibody titers in the range of 10,000 to 100,000 as determined by the reciprocal of the dilution factor which gave 50% of the maximum binding in the ELISA.

To assess whether these monoclonal antibodies bound to the same epitopes, a competitive binding 35 ELISA was performed. At a ratio of biotinylated mAb to unlabeled mAb of 1:100, the binding of biotinylated mAb 5.12.14 was significantly inhibited by its homologous mAb but not by mAb 4.1.3, while the binding of biotinylated mAb 4.1.3 was inhibited by mAb 4.1.3 but not by mAb 5.12.14. Monoclonal antibody 5.2.3 behaved similarly to mAb 4.1.3, while monoclonal antibodies 4.8 and 12.3.9 were similar to mAb 5.12.14. Thus, mAb 4.1.3 and mAb 5.2.3 bind to a different epitope(s) than the epitope recognized by monoclonal antibodies 12.3.9, 4.8 and 5.12.14.

Immunodot blot analysis was performed to assess antibody reactivity to IL-8 immobilized on nitrocellulose paper. All seven antibodies recognized IL-8 immobilized on paper, whereas a control mouse IgG antibody did not.

The ability of these monoclonal antibodies to capture soluble ^{125}I -IL-8 was assessed by a radioimmune precipitation test (RIP). Briefly, tracer ^{125}I -IL-8 (4×10^4 counts per minute (cpm)) was incubated with various dilutions of the monoclonal anti-IL-8 antibodies in 0.2 ml of PBS containing 0.5% BSA and 0.05% Tween 20 (assay buffer) for 1 hr at room temperature. One hundred microliters of a predetermined concentration of goat anti-mouse Ig antisera (Pel-Freez, Rogers, AR) were added and the mixture was incubated at room temperature for 1 hr. Immune complexes were precipitated by the addition of 0.5 milliliters (ml) of 6% polyethylene glycol (molecular weight (M.W.) 8000) kept at 4°C. After centrifugation at 2,000 x gravity (g) for 20 min at 4°C, the supernatant was removed by aspiration and the radioactivity remaining in the pellet was counted in a gamma counter. Percent specific binding was calculated as (precipitated cpm - background cpm)/(total cpm - background cpm). Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14 and 12.3.9 captured ^{125}I -IL-8 very efficiently, while antibodies 9.2.4 and 8.9.1 were not able to capture soluble ^{125}I -IL-8 in the RIP even though they could bind to IL-8 coated onto ELISA plates (Table I).

The dissociation constants of these monoclonal antibodies were determined using a competitive binding RIP assay. Briefly, competitive inhibition of the binding each antibody to ^{125}I -IL-8 (20,000-40,000 cpm per assay) by various amounts of unlabeled IL-8 was determined by the RIP described above. The dissociation constant (affinity) of each mAb was determined by using Scatchard plot analysis (Munson, et al., 20 *Anal. Biochem.* 107:220 (1980)) as provided in the VersaTerm-PRO computer program (Synergy Software, Reading, PA). The dissociation constants (K_d 's) of these monoclonal antibodies (with the exception of 9.2.4. and 8.9.1) were in the range from 2×10^{-8} to 3×10^{-10} moles/liter (M). Monoclonal antibody 5.12.14 with a K_d of 3×10^{-10} M showed the highest affinity among all the monoclonal antibodies tested (Table I).

Table I. Characterization of Anti-IL-8 Monoclonal Antibodies

Antibody	%Specific Binding to IL-8	K_d (M)	Isotype	isoelect. point(pI)
4.1.3	58	2×10^{-9}	IgG ₁	4.3-6.1
5.2.3	34	2×10^{-8}	IgG ₁	5.2-5.6
9.2.4	1	-	IgG ₁	7.0-7.5
8.9.1	2	-	IgG ₁	6.8-7.6
4.8	62	3×10^{-8}	IgG _{2a}	6.1-7.1
5.12.14	98	3×10^{-8}	IgG _{2a}	6.2-7.4
12.3.9	86	2×10^{-8}	IgG _{2a}	6.5-7.1

To assess the ability of these monoclonal antibodies to neutralize IL-8 activity, the amount of ^{125}I -IL-8 bound to human neutrophils in the presence of various amounts of culture supernatants and purified monoclonal antibodies was measured. Neutrophils were prepared by using Mono-Poly Resolving Medium (M-PRM) (Flow Lab. Inc., McLean, VA). Briefly fresh, heparinized human blood was loaded onto M-PRM at a 5 ratio of blood to medium, 3.5:3.0, and centrifuged at 300 x g for 30 min at room temperature. Neutrophils enriched at the middle layer were collected and washed once in PBS. Such a preparation routinely contained greater than 95% neutrophils according to the Wright's Giemsa staining. The receptor binding assay was done as follows. 50 microliters (μl) of ^{125}I -IL-8 (5 nanograms/milliliter (ng/ml)) was incubated with 50 μl of unlabeled IL-8 (100 micrograms/milliliter ($\mu\text{g/ml}$)) or monoclonal antibodies in phosphate buffered saline 10 (PBS) containing 0.1% bovine serum albumin (BSA) for 30 min at room temperature. The mixture was then incubated with 100 μl of neutrophils (10^7 cells/ml) for 15 min at 37°C. The ^{125}I -IL-8 bound was separated from the unbound material by loading mixtures onto 0.4 ml of PBS containing 20% sucrose and 0.1% BSA and by centrifugation at 300 x g for 15 min. The supernatant was removed by aspiration and the radioactivity 15 associated with the pellet was counted in a gamma counter.

Monoclonal antibodies 4.1.3, 5.2.3, 4.8, 5.12.14, and 12.3.9 inhibited greater than 85% of the binding of IL-8 to human neutrophils at a 1:25 molar ratio of IL-8 to mAb. On the other hand, monoclonal antibodies 9.2.4 and 8.9.1 appeared to enhance the binding of IL-8 to its receptors on human neutrophils. Since a control mouse IgG also enhanced the binding of IL-8 on neutrophils, the enhancement of IL-8 binding to its receptors by mAb 9.2.4 and 8.9.1 appears to be nonspecific. Thus, monoclonal antibodies, 4.1.3, 5.1.3, 4.8, 5.12.14, 20 and 12.3.9 are potential neutralizing monoclonal antibodies while monoclonal antibodies 8.9.1 and 9.2.4 are non-neutralizing monoclonal antibodies.

The ability of the anti-IL-8 antibodies to block neutrophil chemotaxis induced by IL-8 was tested as follows. Neutrophil chemotaxis induced by IL-8 was determined using a Boyden chamber method (Larsen, et al. Science 243:1464 (1989)). One hundred μl of human neutrophils (10^6 cells per milliliter (cells/ml)) 25 resuspended in RPMI containing 0.1% BSA were placed in the upper chamber and 29 μl of the IL-8 (20 nM) with or without monoclonal antibodies were placed in the lower chamber. Cells were incubated for 1 hr at 37°C. Neutrophils migrated into the lower chamber were stained with Wright's Giemsa stain and counted under the microscope (100x magnification). Approximately 10 different fields per experimental group were examined. Neutralizing monoclonal antibodies 5.12.14 and 4.1.3 blocked almost 70% of the neutrophil 30 chemotactic activity of IL-8 at 1:10 ratio of IL-8 to mAb.

The isoelectric focusing (IEF) pattern of each mAb was determined by applying purified antibodies on an IEF polyacrylamide gel (pH 3-9, Pharmacia) using the Fast gel system (Pharmacia, Piscataway, NJ). The IEF gel was pretreated with pharmalyte containing 1% Triton X100 (Sigma, St. Louis, MO) for 10 min before loading the samples. The IEF pattern was visualized by silver staining according to the instructions 35 from the manufacturer. All of the monoclonal antibodies had different IEF patterns, confirming that they originated from different clones. The pI values for the antibodies are listed in Table I.

All these monoclonal antibodies bound equally well to both (ala-IL-8)77 and (ser-IL-8)72 forms of IL-8. Because IL-8 has greater than 30% sequence homology with certain other members of the platelet factor 4 (PF4) family of inflammatory cytokines such as β -TG (Van Damme et al., Eur. J. Biochem. 181:337(1989);

Tanaka et al., *FEB* 236(2):467 (1988)) and PF4 (Deuel et al., *Proc. Natl. Acad. Sci. U.S.A.* 74:2256 (1977)), they were tested for possible cross reactivity to β -TG and PF4, as well as to another neutrophil activating factor, C5a. No detectable binding to any of these proteins was observed, with the exception of mAb 4.1.3, which had a slight cross reactivity to β -TG.

5 One of the antibodies, mAb 5.12.14, was further studied to determine whether it could block the IL-8 mediated release of elastase by neutrophils. Briefly, human neutrophils were resuspended in Hanks balanced salt solution (Gibco, Grand Island, NY) containing 1.0% BSA, Fraction V (Sigma, St. Louis, MO), 2 mg/ml alpha-D-glucose (Sigma), 4.2 millimoles/liter (mM) sodium bicarbonate (Sigma) and 0.01 M HEPES, pH 7.1 (JRH Bioscience, Lenexa, KS). A stock of cytochalasin B (Sigma) was prepared (5 mg/ml in 10 dimethylsulfoxide (Sigma) and stored at 2-8°C. Cytochalasin B was added to the neutrophil preparation to produce a final concentration of 5 μ g/ml, and incubated for 15 min at 37°C. Human IL-8 was incubated with mAb 5.12.14 (20 μ l), or a negative control antibody, in 1 ml polypropylene tubes (DBM Scientific, San Fernando, CA) for 30 min at 37°C. The final assay concentrations of IL-8 were 50 and 500 nM. The monoclonal antibodies were diluted to produce the following ratios (IL-8:Mab): 1:50, 1:10, 1:2, 1:1, and 15 1:0.25. Cytochalasin B-treated neutrophils were added (100 μ l/tube) and incubated for 2 hours at 25°C. The tubes were centrifuged (210 X g, 2-8°C) for 10 min, and supernatants were transferred to 96 well tissue culture plates (30 μ l/well). Elastase substrate stock, 10 mM methoxysuccinyl-alanyl-alanyl-propyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA) in DMSO was prepared and stored at 2-8°C. Elastase substrate solution (1.2 mM substrate, 1.2 M NaCl (Mallinckrodt, Paris, Kentucky), 0.12 M HEPES pH 7.2 in distilled water) was added 20 (170 μ l/well) to the supernatants and incubated for 0.5 to 2 hours at 37°C (until control optical density (O.D.) of 1.0 was reached). Absorbance was measured at 405 nm (SLT 340 ATTC plate reader, SLT Lab Instruments, Austria).

The results are shown in Figure 1. At a 1:1 ratio of IL-8 to mAb 5.12.14, the antibody was able to effectively block the release of elastase from neutrophils.

25 The hybridoma producing antibody 5.12.14 was deposited on February 15, 1993 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. HB 11553. This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).

30 B. GENERATION AND CHARACTERIZATION OF MONOCLONAL ANTIBODIES AGAINST RABBIT IL-8

Antibodies against rabbit IL-8 were generated in essentially the same process as anti-human IL-8 antibodies using rabbit IL-8 as immunogen (kindly provided by C. Broaddus; see also Yoshimura et al. *J. Immunol.* 146:3483 (1991)). The antibody was characterized as described above for binding to other cytokines 35 coated onto ELISA plates; no measurable binding was found to MGSA, fMLP, C5a, β -TG, TNF, PF4, or IL-1.

The hybridoma producing antibody 6G4.2.5 was deposited on September 28, 1994, with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. HB 11722. This deposit was made under the provisions of the Budapest Treaty on the

International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).

Recombinant human-murine chimeric Fabs for 5.12.14 and 6G4.2.5 were constructed as described below. A chimeric 6G.4.25 Fab is compared with a chimeric 5.12.14 Fab in detail below.

5 I. Inhibition of IL-8 binding to human neutrophils by 5.12.14-FAB and 6G4 2.5-FAB

The ability of the two chimeric Fabs, 5.12.14-Fab and 6G4.2.5-Fab, to efficiently bind IL-8 and prevent IL-8 from binding to IL-8 receptors on human neutrophils was determined by performing a competition binding assay which allows the calculation of the IC_{50} - concentration required to achieve 50% inhibition of IL-8 binding.

10 Human neutrophils (5×10^5) were incubated for 1 hour at 4°C with 0.5nM ^{125}I -IL-8 in the presence of various concentrations (0 to 300 nM) of 5.12.14-Fab, 6G4.2.5-Fab, an isotype control (4D5-Fab) or unlabeled IL-8. After the incubation, the unbound ^{125}I -IL-8 was removed by centrifugation through a solution of 20% sucrose and 0.1% bovine serum albumin in phosphate buffered saline and the amount of ^{125}I -IL-8 bound to the cells was determined by counting the cell pellets in a gamma counter. Figure 2 demonstrates the 15 inhibition of ^{125}I -IL-8 binding to neutrophils by unlabeled IL-8. Figure 3 demonstrates that a negative isotype matched Fab does not inhibit the binding of ^{125}I -IL-8 to human neutrophils. Both the anti-IL-8 Fabs, 5.12.14 Fab (Figure 4) and 6G.4.25 Fab (Figure 5) were able to inhibit the binding of ^{125}I -IL-8 to human neutrophils with an average IC_{50} of 1.6 nM and 7.5 nM, respectively.

II. Inhibition of IL-8-mediated neutrophil chemotaxis by 5.12.14-FAB and 6G4.2.5-FAB

20 Human neutrophils were isolated, counted and resuspended at 5×10^6 cells/ml in Hank's balanced salt solution (abbreviated HBSS; without calcium and magnesium) with 0.1% bovine serum albumin. The neutrophils were labeled by adding calcein AM (Molecular Probe, Eugene, OR) at a final concentration of 2.0 micromoles/liter (μ M). Following a 30 minute incubation at 37°C, cells were washed twice with HBSS-BSA and resuspended at 5×10^6 cells/ml.

25 Chemotaxis experiments were carried out in a Neuro Probe (Cabin John, MD) 96-well chamber, model MBB96. Experimental samples (buffer only control, IL-8 alone or IL-8 + Fabs) were loaded in a Polyfiltronics 96-well View plate (Neuro Probe Inc.) placed in the lower chamber. 100 μ l of the calcein AM-labeled neutrophils were added to the upper chambers and allowed to migrate through a 5 micrometer porosity PVP free polycarbonate framed filter (Neuro Probe Inc.) toward the bottom chamber sample. The chemotaxis 30 apparatus was then incubated for 40 to 60 minutes at 37°C with 5% CO_2 . At the end of the incubation, neutrophils remaining in the upper chamber were aspirated and upper chambers were washed three times with PBS. Then the polycarbonate filter was removed, non-migrating cells were wiped off with a squeegee wetted with PBS, and the filter was air dried for 15 minutes.

35 The relative number of neutrophils migrating through the filter (Neutrophil migration index) was determined by measuring fluorescence intensity of the filter and the fluorescence intensity of the contents of the lower chamber and adding the two values together. Fluorescence intensity was measured with a CytoFluor

2300 fluorescent plate reader (Millipore Corp. Bedford, MA) configured to read a Corning 96-well plate using the 485-20 nm excitation filter and a 530-25 emission filter, with the sensitivity set at 3.

5 The results are shown in Figures 6 and 7. Figure 6 demonstrates the inhibition of human IL-8 mediated neutrophil chemotaxis by chimeric 6G4.2.5 and 5.12.14 Fabs. Figure 7 demonstrates the relative abilities of chimeric 6G4.2.5 and 5.12.14 Fabs to inhibit rabbit IL-8 mediated neutrophil chemotaxis.

III. Inhibition of IL-8-mediated neutrophil elastase release by various concentrations of 6G4.2.5 AND 5.12.14 FABS

10 Blood was drawn from healthy male donors into heparinized syringes. Neutrophils were isolated by dextran sedimentation, centrifugation over Lymphocyte Separation Medium (Organon Teknika, Durham, NC), and hypotonic lysis of contaminating red blood cells as described by Berman et al. (*J. Cell Biochem.* 52:183 (1993)). The final neutrophil pellet was suspended at a concentration of 1×10^7 cells/ml in assay buffer, which consisted of Hanks Balanced Salt Solution (GIBCO, Grand Island, NY) supplemented with 1.0% BSA (fraction V, Sigma, St. Louis, MO), 2 mg/ml glucose, 4.2 mM sodium bicarbonate, and 0.01 M HEPES, pH 7.2. The neutrophils were stored at 4°C for not longer than 1 hr.

15 15 IL-8 (10 μ l) was mixed with anti-IL-8 Fab, an isotype control Fab, or buffer (20 μ l) in 1 ml polypropylene tubes and incubated in a 37°C water bath for 30 min. IL-8 was used at final concentrations ranging from 0.01 to 1000 nM in dose response studies (Figure 8) and at a final concentration of 100 nM in the experiments addressing the effects of the Fabs on elastase release (Figures 9 and 10). Fab concentrations ranged from approximately 20 nM to 300 nM, resulting in Fab:IL-8 molar ratios of 0.2:1 to 3:1. Cytochalasin B (Sigma) was added to the neutrophil suspension at a concentration of 5 μ g/ml (using a 5 mg/ml stock solution made up in DMSO), and the cells were incubated for 15 min in a 37°C water bath. Cytochalasin B-treated neutrophils (100 μ l) were then added to the IL-8/Fab mixtures. After a 3 hr incubation at room temperature, the neutrophils were pelleted by centrifugation (200 x g for 5 min), and aliquots of the cell-free supernatants were transferred to 96 well plates (30 μ l/well). The elastase substrate, methoxysuccinyl-alanyl-alanyl-prolyl-valyl-p-nitroanilide (Calbiochem, La Jolla, CA), was prepared as a 10 mM stock solution in DMSO and stored at 4°C. Elastase substrate working solution was prepared just prior to use (1.2 mM elastase substrate, 1.2 M NaCl, 0.12 M HEPES, pH 7.2), and 170 μ l was added to each sample-containing well. The plates were placed in a 37°C tissue culture incubator for 30 min or until an optical density reading for the positive controls reached at least 1.0. Absorbance was measured at 405 nm using an SLT 340 plate reader (SLT Lab Instruments, Austria).

20 25 30

Figure 9 demonstrates the ability of the chimeric anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by human IL-8; Figure 10 demonstrates the relative abilities of the chimeric anti-IL-8 Fabs to inhibit elastase release from human neutrophils stimulated by rabbit IL-8.

C. EXPERIMENTAL COLITIS MODEL

35 One of the most widely accepted models of chronic experimental colitis is 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced injury, recently described by Morris et al., *Gastroenterology* 96:795 (1989). Briefly, rectal administration of 10 to 30 milligrams (mg) of TNBS in 0.25 ml of 50% ethanol

produces acute and chronic local inflammation documented by dose-dependent increases in colonic weights, gross ulceration, and myeloperoxidase values. High doses of TNBS (30 mg) in ethanol produces colonic injury that peaks at 1 week but persists for at least 8 weeks after administration. Colonic inflammation is accompanied by weight loss in the first week, diarrhea in 90% of animals during weeks 1 to 3, and stenosis of 5 the distal colon with proximal dilation, but only 3% mortality. In chronic phases, inflammation is segmental with linear (transverse) ulcers and marked thickening of the colon. Transmural acute and chronic inflammation is noted histologically with a progressive increase in inflammatory cell infiltration in the external muscle and serosa during weeks 3 to 5. Mucosal and serosal granulomas are present in 55% of animals examined at 2 to 3 weeks and in approximately 20% of animals 4 weeks or more after injury.

10 To study the ability of the anti-IL-8 antibodies of the invention to attenuate acute colitis in rabbits, colitis was induced in New Zealand White rabbits (1.8-2 kilograms (kg) body weight) by intracolonic instillation of 5 ml of 17-35 mg/ml Trinitrobenzene sulfonic acid in 30% ethanol (TNBS/EtOH) (adapted from the method of Morris et al., *Gastroenterology* 96:795 (1989)). Five rabbits were treated intravenously with 5 mg/kg 6G4.2.5. Three control rabbits received PBS. Animals treated with TNBS/EtOH were euthanized after 15 24 hours post dosing and the colon tissue was examined for levels of IL-8, myeloperoxidase (enzyme marker for polymorphonuclear leukocytes or heterophils), wet colon weight, gross inflammation, and histopathology. Two sections of colon were preserved in formalin, processed by standard procedures for routine hematoxylin and eosin sections. The colon tissue was examined for levels of IL-8 by enzyme linked immunoassay. Wet colon weight from treated and untreated rabbits was measured and compared. Edema was measured as the 20 thickness of the submucosa in 3 to 5 sites per sample. Leukocytic margination was evaluated by determining which vessels in the tissue section were affected (e.g., superficial, involving only the subepithelial vessels in the lamina propria, to marked, involving vessels in the submucosa). The extent of necrosis was measured as the percent of the colon manifesting necrosis. The severity of necrosis was measured as the depth of penetration of necrosis into the wall of the colon. Gross inflammation was defined as the severity of 25 inflammation over the length of the involved colon and was scored visually based upon the degree of swelling and coloration. Leukocytic infiltration was determined by counting the number of neutrophils per high power field (HPF) (40X magnification). Mononuclear cell infiltration was determined by counting the number of mononuclear cells per HPF (40X magnification).

30 Heterophil (neutrophil) influx into inflamed rabbit colonic tissue was monitored by measurement of MPO levels (see, for example, Bradley et al., *J. Invest. Dermatol.* 7B:206 (1982)). Briefly, colonic sections were placed in 15 ml polypropylene tubes and incubated at 60°C for 2 hours. The tissues were frozen in liquid nitrogen. Fine powder tissue lysates were prepared with a mortar and pestle and transferred into 15 ml polypropylene tubes. The tissue samples were solubilized in 0.5% hexadecyl trimethyl ammonium (HTAB) (0.5% weight to volume (w/v) in 50mM KPO₄ buffer at pH6) at a ratio of 3.5 ml per gram of tissue using a 35 tissue homogenizer. The samples were frozen and thawed twice by freezing in liquid nitrogen and thawing in 60°C water bath. The samples were then sonicated for 10 seconds at a 50% duty cycle at 2.5 power level. Each sample lysate was transferred to an microfuge tube and centrifuged at room temperature for 15 minutes at 15,600 x g. The samples were transferred to fresh clean Microfuge tubes. Seventy five μ l of each sample and 75 μ l of human MPO standard positive control (Calbiochem Corp., San Diego, CA) in HTAB diluted to 0.03

units per well were transferred in triplicate to a 96 well flat bottom plate. Seventy-five μ l of HTAB (0.5% w/v in 50mM KPO₄ buffer pH 6.0) were added as reference blanks. One hundred μ l of H₂O₂ were added to each well. The reaction in the 96 well plate was monitored on a Thermo Max optical plate reader (Molecular Devices Co. Menlo Park, CA). A stock solution of O-dianisidine (Sigma, St. Louis, MO) at 10 mg dry powder 5 in 1.0 ml of distilled H₂O was prepared and drawn through a 0.2 micron filter. Twenty-five μ l were added to each well. The plates were read at OD 450 nm continuously at 3-5 minute intervals over a 30 minute period.

Increased levels of myeloperoxidase and IL-8 were detected in animals dosed with increasing doses of TNBS/EtOH as compared to sham treated control animals. Increased colonic weight and gross inflammation were also evident. Histological evaluation revealed mucosal necrosis of the bowel wall, with heterophil 10 margination of the blood vessels and infiltration in the affected tissue.

However, treatment of rabbits with anti-IL-8 antibodies reduced the severity of TNBS/EtOH-induced colitis. Lesions in animals treated with 5 milligrams per kilogram of body weight (mg/kg) intravenous 6G4.2.5, just prior to colitis induction with TNBS/EtOH, were attenuated in 4 of 5 animals as compared to 3 control animals. Antibody treatment reduced the extent and severity of necrosis, gross inflammation, colonic weight, 15 edema, heterophil margination and infiltration. The levels of colonic myeloperoxidase and IL-8 were greatly reduced. The results of these experiments are depicted in Figure 11. These observations support the usefulness of anti-IL-8 antibodies in the attenuation of colitis.

D. EFFECT OF ANTI-IL-8 ON NEUTROPHIL EMIGRATION DURING BACTERIAL PNEUMONIA

Neutrophils migrate into the lung in response to a variety of stimuli, including infection by 20 Streptococcus pneumoniae. To determine whether the anti-IL-8 antibodies of the instant invention could inhibit such neutrophil migration, thereby ameliorating inflammation in the lung, a rabbit pneumonia model was used. Briefly, anesthetized New Zealand white rabbits were given intrabronchial instillations of Streptococcus pneumoniae, Escherichia coli, or Pseudomonas aeruginosa (3×10^9 organisms/ml) combined with either anti-rabbit IL-8 antibody (clone 6G4.2.5) or control mouse IgG (final concentration 0.5 mg/ml) and colloidal carbon 25 (5%) in a total volume of 0.5 ml. After 3 hours and 50 min, the rabbits received an intravenous injection of radiolabeled microspheres to measure pulmonary blood flow. At 4 hours, the heart and lungs were removed and the lungs were separated. The pneumonic region (usually the left lower lobe) as indicated by the colloidal carbon and the corresponding region in the contralateral lung was lavaged using phosphate-buffered saline. Total leukocyte counts were obtained using a hemacytometer on the lavage fluid and differential counts were 30 performed on Wright-stained cytocentrifuge preparations.

Treatment with anti-rabbit IL-8 antibodies significantly reduced the number of neutrophils present in the BAL fluid compared to animals treated with isotype control mouse IgG (Figure 12). Thus, anti-IL-8 antibodies effectively reduce neutrophil emigration in the pneumonic lung.

E. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE 35 MURINE 5.12.14 (ANTI-IL-8) MONOCLONAL ANTIBODY

Total RNA was isolated from 1×10^8 cells (hybridoma cell line ATCC HB-11722) using the procedure described by Chomczynski and Sacchi (Anal. Biochem. 162:156 (1987)). First strand cDNA was

synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest, Kabat, E. A. et al. (1991) NIH Publication 91-3242, V 1-3.). Three primers were designed for each of the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 13). Amplification of the first strand cDNA to double-stranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer and one reverse primer for the light chain variable region amplification (Figure 14) and one forward primer and one reverse primer for the heavy chain variable region amplification (Figure 15). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 5.12.14 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids was sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, *M*luI, for both the light chain variable region forward primer and the heavy chain variable region forward primer to facilitate ligation to the 3' end of the STII element in the cloning vector. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique *B*stBI restriction site and the heavy chain variable region reverse primer contained a unique *A*paI restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vectors, pB13.1 (light chain) and pB14 (heavy chain). The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp. The cDNA encoding the 5.12.14 light chain variable region was cloned into the vector pB13.1, to form pA51214VL and the 5.12.14 heavy chain variable region was cloned into the vector, pB14, to form pA51214VH. The cDNA inserts were characterized by DNA sequencing and are presented in Figure 16 (murine light chain variable region) and Figure 17 (murine heavy chain variable region).

F. CONSTRUCTION OF A 5.12.14 FAB VECTOR

In the initial construct, pA51214VL, the amino acids between the end of the 5.12.14 murine light chain variable sequence and the unique cloning site, *B*stBI, in the human IgG1 constant light sequence were of murine origin corresponding to the first 13 amino acids of the murine IgG1 constant region (Figure 16). Therefore, this plasmid contained a superfluous portion of the murine constant region separating the 5.12.14 murine light chain variable region and the human light chain IgG1 constant region. This intervening sequence would alter the amino acid sequence of the chimera and most likely produce an incorrectly folded Fab. This problem was addressed by immediately truncating the cDNA clone after A109 and re-positioning the *B*stBI site to the variable/constant junction by the polymerase chain reaction. Figure 18 shows the amplification primers used to make these modifications. The forward primer, VL.front, was designed to match the last five amino acids of the STII signal sequence, including the *M*luI cloning site, and the first 4 amino acids of the 5.12.14 murine light chain variable sequence. The sequence was altered from the original cDNA in the third

position of the first two codons D1 (T to C) and I2 (C to T) to create a unique EcoRV cloning site which was used for later constructions. The reverse primer, VL.rear, was designed to match the first three amino acids of the human IgG1 constant light sequence and the last seven amino acids of the 5.12.14 light chain variable sequence which included a unique BstBI cloning site. In the process of adding the BstBI site, the nucleotide sequence encoding several amino acids were altered: L106 (TTG to CTT), K107 (AAA to CGA) resulting in a conservative amino acid substitution to arginine, and R108 (CGG to AGA). The PCR product encoding the modified 5.12.14 light chain variable sequence was then subcloned into pB13.1 in a two-part ligation. The MluI-BstBI digested 5.12.14 PCR product encoding the light chain variable region was ligated into MluI-BstBI digested vector to form the plasmid, pA51214VL'. The modified cDNA was characterized by DNA sequencing. The coding sequence for the 5.12.14 light chain is shown in Figure 19.

Likewise, the DNA sequence between the end of the heavy chain variable region and the unique cloning site, ApaI, in the human IgG1 heavy chain constant domain of pA51214VH was reconstructed to change the amino acids in this area from murine to human. This was done by the polymerase chain reaction. Amplification of the murine 5.12.14 heavy chain variable sequence was accomplished using the primers shown in Figure 18. The forward PCR primer was designed to match nucleotides 867-887 in pA51214VH upstream of the STII signal sequence and the putative cDNA sequence encoding the heavy chain variable region and included the unique cloning site SpeI. The reverse PCR primer was designed to match the last four amino acids of the 5.12.14 heavy chain variable sequence and the first six amino acids corresponding to the human IgG1 heavy constant sequence which also included the unique cloning site, ApaI. The PCR product encoding the modified 5.12.14 heavy chain variable sequence was then subcloned to the expression plasmid, pMHM24.2.28 in a two-part ligation. The vector was digested with SpeI-ApaI and the SpeI-ApaI digested 5.12.14 PCR product encoding the heavy chain variable region was ligated into it to form the plasmid, pA51214VH'. The modified cDNA was characterized by DNA sequencing. The coding sequence for the 5.12.14 heavy chain is shown in Figure 20.

The first expression plasmid, pantill-8.1, encoding the chimeric Fab of 5.12.14 was made by digesting pA51214VH' with EcoRV and Bpu1102I to replace the EcoRV-Bpu1102I fragment with a EcoRV-Bpu1102I fragment encoding the murine 5.12.14 light chain variable region of pA51214VL'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

Preliminary analysis of Fab expression using pantill-8.1 showed that the light and heavy chains were produced intracellularly but very little was being secreted into the periplasmic space of E. coli. To correct this problem, a second expression plasmid was constructed.

The second expression plasmid, pantill-8.2, was constructed using the plasmid, pmy187, as the vector. Plasmid pantill-8.2 was made by digesting pmy187 with MluI and SphI and the MluI (partial)-SphI fragment encoding the murine 5.12.14 murine-human chimeric Fab of pantill-8.1 was ligated into it. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 5.12.14.

The plasmid pantill-8.2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATTC Accession No. ATCC

97056. This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).

5 G. MOLECULAR CLONING OF THE VARIABLE LIGHT AND HEAVY REGIONS OF THE
5 MURINE 6G4.2.5 MONOCLONAL ANTIBODY

Total RNA was isolated from 1×10^8 cells (hybridoma cell line 6G4.2.5) using the procedure described by Chomczynski and Sacchi (*Anal. Biochem.* 162:156 (1987)). First strand cDNA was synthesized by specifically priming the mRNA with synthetic DNA oligonucleotides designed to hybridize with regions of the murine RNA encoding the constant region of the kappa light chain or the IgG2a heavy chain (the DNA sequence of these regions are published in Sequences of Proteins of Immunological Interest, Kabat et al. (1991) NIH Publication 91-3242, V 1-3). Three primers were designed for each the light and heavy chains to increase the chances of primer hybridization and efficiency of first strand cDNA synthesis (Figure 21). Amplification of the first strand cDNA to double-stranded (ds) DNA was accomplished using two sets of synthetic DNA oligonucleotide primers: one forward primer and one reverse primer for the light chain variable region amplification (Figure 22) and one forward primer and one reverse primer for the heavy chain variable region amplification (Figure 23). The N-terminal sequence of the first eight amino acids of either the light or heavy chains of 6G4.2.5 was used to generate a putative murine DNA sequence corresponding to this region. (A total of 29 amino acids were sequenced from the N-terminus of both the light chain and heavy chain variable regions using the Edman degradation protein sequencing technique.) This information was used to design the forward amplification primers which were made degenerate in the third position for some codons to increase the chances of primer hybridization to the natural murine DNA codons and also included the unique restriction site, Nsil, for the light chain variable region forward primer and the unique restriction site, MluI, for the heavy chain variable region forward primer to facilitate ligation to the 3' end of the STII element in the vector, pchimFab. The reverse amplification primers were designed to anneal with the murine DNA sequence corresponding to a portion of the constant region of the light or heavy chains near the variable/constant junction. The light chain variable region reverse primer contained a unique MunI restriction site and the heavy chain variable region reverse primer contained a unique Apal restriction site for ligation to the 5' end of either the human IgG1 constant light or IgG1 constant heavy regions in the vector, pchimFab. The polymerase chain reaction using these primer sets yielded DNA fragments of approximately 400 bp and were cloned individually into the vector, pchimFab, to form p6G425VL and p6G425VH. The cDNA inserts were characterized by DNA sequencing and are presented in Figure 24 (murine light chain variable region) and Figure 25 (murine heavy chain variable region).

25 H. CONSTRUCTION OF A 6G4.2.5 CHIMERIC FAB VECTOR

In the initial construct, p6G425VL, the amino acids between the end of the 6G4.2.5 murine light chain variable sequence and the unique cloning site, MunI, in the human IgG1 constant light sequence were of murine origin. These amino acids must match the human IgG1 amino acid sequence to allow proper folding of the chimeric Fab. Two murine amino acids, D115 and S121, differed dramatically from the amino acids

found in the loops of the β -strands of the human IgG1 constant domain and were converted to the proper human amino acid residues, V115 and F121, by site-directed mutagenesis using the primers shown in Figure 26. These specific mutations were confirmed by DNA sequencing and the modified plasmid named p6G425VL'. The coding sequence is shown in Figure 27.

5 Likewise, the DNA sequence between the end of the heavy chain variable region and the unique cloning site, Apal, in the human IgG1 heavy chain constant domain of p6G425VH was reconstructed to change the amino acids in this area from murine to human. This process was facilitated by the discovery of a BstEII site near the end of the heavy chain variable region. This site and the Apal site were used for the addition of a synthetic piece of DNA encoding the corresponding IgG human amino acid sequence. The
10 synthetic oligo-nucleotides shown in Figure 26B were designed as complements of one another to allow the formation of a 27 bp piece of ds DNA. The construction was performed as a three-part ligation because the plasmid, p6G425VH, contained an additional BstEII site within the vector sequence. A 5309 bp fragment of p6G425VH digested with MluI-Apal was ligated to a 388 bp fragment carrying the 6G4.2.5 heavy chain variable region and a 27 bp synthetic DNA fragment encoding the first six amino acids of the human IgG1
15 constant region to form the plasmid, p6G425VH'. The insertion of the synthetic piece of DNA was confirmed by DNA sequencing. The coding sequence is shown in Figure 28.

The expression plasmid, p6G425chim2, encoding the chimeric Fab of 6G4.2.5 was made by digesting p6G425chimVL' with MluI and Apal to remove the STII-murine HPC4 heavy chain variable region and replacing it with the MluI-Apal fragment encoding the STII-murine 6G4.2.5 heavy chain variable region of p6G425chimVH'. The resultant plasmid thus contained the murine-human variable/constant regions of both the light and heavy chains of 6G4.2.5.

20 The plasmid p6G425chim2 was deposited on February 10, 1995 with the American Type Culture Collection, 12301 Parklawn Drive, Rockville, MD, U.S.A. (ATCC) and assigned ATCC Accession No. 97055. This deposit was made under the provisions of the Budapest Treaty on the International Recognition of the
25 Deposit of Microorganisms for the Purpose of Patent Procedure and the Regulations thereunder (Budapest Treaty).

I. EFFECT OF IL-8 HOMOLOG RECEPTOR KNOCK-OUT IN TRANSGENIC MOUSE ASTHMA MODEL

30 IL-8 is a potent chemoattractant for neutrophils and has also been shown to activate eosinophils. To investigate the function of IL-8 in the migration and activation of leukocytes in asthma, a murine model for allergic asthma utilizing wild type and IL-8 homolog receptor knock-out mice was developed. The murine asthma model reproduces several aspects typical of this disease, including allergen-specific IgE titers, high percentage of eosinophils in the bronchoalveolar lavage, lung mucosal infiltrates of macrophages, lymphocytes and plasma cells, and hypersecretion of mucus.

35 Balb/C IL-8 homolog receptor knock-out mice were generated by crossing C57BL/6J IL-8 homolog receptor (IL8Rh) knock-out mice (produced according to the methods of Cacalano *et al.*, *Science*, 265: 682-684 (1994)) against balb/C mice until a genotype consisting of the IL8Rh knock-out carrying chromosome against a balb/C genetic background was obtained. Female Balb/C wild type (WT) and IL-8 homolog receptor knock-

out (KO) litter mates were bred and checked for genotype by tail sampling. The animals were 6 to 8 weeks old at the beginning of the study.

Both WT and KO mice were randomly divided into groups of controls and asthmatics, each group containing 7 animals for statistical analysis. The asthmatic groups were injected intraperitoneally on day 0 with 5 0.1 ml of a solution of 100 μ g/ml ovalbumin grade V (Sigma, MO) and 10 mg/ml aluminum oxide (Intergen, NY) in Dulbecco's Phosphate Buffered Saline (DPBS) (HyClone, UT). On day 14 through day 20 both asthmatic and control groups were aerosolized for 30 minutes each day with a solution of 10 mg/ml ovalbumin in DPBS. The aerosolization was performed by placing 14 animals in a 16.5x17x52cm Plexiglas cage connected to a Ultra-Neb 99 nebulizer (DeVilbiss, PA) set at an output of 1.7 ml/min. Serum, whole blood, 10 bronchoalveolar lavage and lung tissues were harvested on day 21 as described below.

Blood was collected through the orbital sinus and clotted in a Microtainer Serum Separator (Becton Dickinson, NJ) for serum harvest.

Ovalbumin-specific IgE titers were determined as follows. Wells in Maxi-Sorp F96 Nunc-Immunoplates (Nunc, Denmark) were each coated with 100 μ l of 2 μ g/ml Fc ϵ RI-HuIgG1 (obtained as 15 described in Haak-Frendscho *et al.*, *J. Immunol.*, 151: 351-358 (1993)) in phosphate buffered saline (PBS) and incubated overnight at 4°C. Plates were rinsed twice in PBS and coated wells were each incubated for 1-2 hours at room temperature (RT) in 400 μ l of blocking solution (50 mM Tris-buffered saline, 0.5% bovine 20 serum albumin (BSA), 0.05% Tween 20 in PBS). Serum samples were serially diluted (beginning with a 1:20 dilution) in blocking solution, and each dilution was layered onto a coated well. The plates were incubated at RT for two hours with agitation.

Following the incubation of the coated plates with serum samples, the plates were rinsed 3 times in a washing buffer and each well was incubated with 100 μ l of 10 μ g/ml ovalbumin in blocking solution for 1 hour at RT. Goat anti-ovalbumin (Cappel (Organon Teknika) Catalog# 55297, Durham, NC) was conjugated to horseradish peroxidase (HRP) and diluted 1:7000 in blocking solution. Plates were then rinsed 3 times in 25 washing buffer and each well was incubated with 100 μ l of the HRP-conjugated goat anti-ovalbumin dilution for 1 hour at RT with agitation. Plates were again rinsed 3 times in washing buffer and each well was developed in 100 μ l o-phenylenediamine dihydrochloride (OPD) solution (mixed from one 5 mg OPD tablet (Sigma), 12.5 ml PBS, and 5 μ l H₂O₂) and 100 μ l 2 M H₂SO₄ for 30 minutes at RT. The plates were assayed for fluorescence at 492 nm in a UV Kinetic Microplate Reader (Molecular Devices, CA).

30 Whole blood was collected through the orbital sinus into 0.2% K₂EDTA and checked for clots. Blood smears of each animal were air dried, fixed in methanol and stained with Diff-Quick (Baxter, IL). Microscopic examination of these slides determined the eosinophil, macrophage, lymphocyte, neutrophil and basophil differentials. Hemograms were obtained by analyzing 10 ml of a 1:250 dilution of whole blood on a Serono 9018 Hematology Analyzer (Baker Diagnostics, NJ). Calculation of the total number of cells was done by 35 assuming that 7% of the mouse body weight is blood.

Mice were anesthetized with 0.1 mg/kg Ketamine HCl (Ketaset, Fort Dodge Laboratories, IO) and 0.5 mg/kg Acepromazine Maleate (PromAce, Aveco Co. Inc., IO) delivered in a single intraperitoneal injection. The mice were placed in dorsal recumbency and the trachea surgically exposed and incised 1/2 to 2/3 through to insert a cannula. The cannula (Micro-renathane, 0.040 OD x 0.025 ID) was connected to a blunt

22 gauge needle and this was attached to a three-way stopcock assembled with two 3cc syringes. One syringe contained 2 ml HBSS (BioWhittaker, MD) and the other syringe was empty for collection. The lungs were gently lavaged with 4 x 0.5 ml aliquots of HBSS, which were collected into the empty syringe. Once harvested, the lavages were kept refrigerated.

5 The cells in the lavages were pelleted and resuspended in 0.2 ml saline. Hemograms were obtained as described above and the concentration adjusted to 200-400 cells/ μ l. An aliquot of 150 μ l was utilized to prepare a slide using a Shandon Cytospin 3 centrifuge. The slides were dried, fixed, stained and read as described above for the differential.

10 The mice were euthanized by cervical dislocation after the lavages were collected and the lungs were surgically removed. A 3cc syringe fitted with a 22 gauge blunt needle was filled with 10% neutral buffered formalin pH 6.8-7.2 (Richard-Allan, MI) and inserted into the trachea. The lungs were gently inflated and the trachea sutured. The tissue specimens were stored in the 10% buffered formalin for further processing.

15 Lung tissue specimens were prepared by cutting a longitudinal section of the left lobe and a cross section of the 3 right lobes, processing the samples in a TissueTek VIP (Miles, NY) to exchange water for paraffin, embedding each sample in a paraffin cube, obtaining thin sections by microtome cutting (Leica, Germany), mounting the thin sections on slides, and staining the mounted samples with hematoxylin-eosin and sealing with a cover glass.

20 As shown in the eosinophil bronchoalveolar lavage (BAL) counts obtained for asthmatic IL8Rh KO mice and asthmatic WT mice displayed in Table II below and in Figure 29, the asthmatic KO mice presented a dramatic 18-fold decrease in the number of eosinophils in the bronchoalveolar lavage when compared to WT asthmatics.

Table II. muIL8Rh regulation of leukocyte populations upon allergic challenge

cell type	mouse genotype	Cir. Blood Cells	Infiltr. cells (BAL)	Infiltr. cell % of Cir.cell	WT:KO ratio
25 eos	WT	1293566	422348	32.65	1.97
	KO	336697	55877	16.60	
lympho	WT	6596926	156121	2.37	2.47
	KO	7247362	69341	0.96	
neutro	WT	2442790	12529	0.51	18.31
	KO	4190160	1174	0.03	

The ovalbumin-specific IgE titers in control and asthmatic animal sera (Figure 32) confirmed that all the animals in the asthmatic group had been sensitized to ovalbumin during the course of the daily ovalbumin aerosol exposure. Thus, the reduced eosinophil response observed in KO asthmatics was not due to the 30 absence of allergen sensitization. Since eosinophils are known to contribute to the pathogenesis of asthma by synthesizing leukotriene C4, stimulating histamine release from mast cells and basophils and releasing the major basic protein, the reduced eosinophil response is indicative of an improvement in the overall pathology of the asthmatic mouse lung.

35 The deletion of the IL8Rh can reduce the lung eosinophil infiltration by directly affecting transmigration of cells into the lung and by indirectly regulating the proliferation and/or circulating half-life

of eosinophils. As shown in Figure 30, the peripheral blood eosinophil count in the asthmatic WT animals increased 24-fold upon repeated stimulation with allergen, while the KO animals exhibited a 3-fold increase. Since human asthmatics also present elevated levels of circulating eosinophils, these data strongly indicate that IL-8 plays a pivotal role in the development of eosinophilia in humans.

5 As shown in Table II above and in Figure 31, the KO mice in comparison to WT mice exhibited a two fold reduction in the ratio of infiltrating versus circulating eosinophils. This is a strong indication that the murine IL8Rh (muIL8Rh) is directly involved in allowing eosinophils to move across the endothelium and epithelium of the lung into the lumen of the airways. This transmigration impairment was also observed for lymphocytes and neutrophils. As shown in Table II above, asthmatic IL8Rh KO mice presented a 2.5-fold and
10 18-fold reduction in the number of lymphocytes and neutrophils, respectively, in the bronchoalveolar lavage when compared to WT asthmatics. These data are of particular significance in light of the fact that human lymphocytes and neutrophils are known to have IL-8 receptors and eosinophils from asthmatic human donors are known to display IL-8 receptors as well.

15 The overall effect of muIL8Rh in the leukocyte infiltration response is clear from the microscopic analysis of lung sections shown in Figures 33-41. Even though the KO mice still presented a mild pulmonary infiltration of leukocytes, the overall severity was greatly reduced compared to WT animals, which were on average moderately and even markedly infiltrated. Even in the less severe WT asthmatics, almost every bronchus and blood vessel was peripherally infiltrated to some extent with monocytes and eosinophils (shown in Figures 36-38), while in the most affected KO animal only the major branches were affected (shown in Figures 39-41).

20 Since this murine asthma model reproduces many of the key physiological features of human allergic asthma and since the muIL8Rh plays such a dramatic role in the development of several asthma symptoms, these data strongly indicate that IL-8 plays an important role in the pathogenesis of asthma in humans, especially with regard to the control of the leukocyte infiltration response. The use of an IL-8 antagonist can
25 reduce the pool of circulating eosinophils to close to baseline levels and improve the overall pathology of the lung by diminishing the number of infiltrating eosinophils and lymphocytes. Since eosinophils stimulate mast cells and basophils to release histamine, which is responsible for inducing smooth muscle contraction and consequently bronchoconstriction, treatment with IL-8 antagonists is expected to decrease the intensity and/or the frequency of airflow obstruction and improve the overall lung function of asthmatic patients.

30 The treatment of asthma with an IL-8 antagonist is investigated by using a primate asthma model in which asthma is induced by intraperitoneal injection of allergen followed by aerosolization with allergen using a protocol similar to that of the murine asthma model described above. The allergen used is ovalbumin or any other antigen known to cause allergy in humans such as dust mite, ragweed, cat dander, etc. A prophylactic treatment modality is investigated by pretreating animals with anti-IL-8 antibody administered intravenously
35 in a single bolus dosage of about 0.1 to 10 mg/kg (or with a small molecule IL-8 antagonist administered intravenously at a dosage to be determined according to the pharmacodynamic profile of the compound) up to about 10 minutes prior to the induction of asthma by aerosolization with allergen as described above. Pretreatment with IL-8 antagonist is expected to prevent or reduce the onset of symptoms resulting from the induction of asthma. Similarly, a therapeutic treatment modality is investigated by inducing asthma in animals

as described above, and treating the animals following onset of asthma with an anti-IL-8 antibody administered intravenously in a single bolus dosage of about 0.1 to 10 mg/kg (or with a small molecule IL-8 antagonist administered intravenously at a dosage to be determined according to the pharmacodynamic profile of the compound). Therapeutic treatment with IL-8 antagonist after onset is expected to reduce or eliminate 5 symptoms resulting from the induction of asthma.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

- (i) APPLICANT: Genentech, Inc.
- 5 (ii) TITLE OF INVENTION: IL-8 Antagonists for Treatment of Inflammatory Disorders and Asthma
- (iii) NUMBER OF SEQUENCES: 58
- 10 (iv) CORRESPONDENCE ADDRESS:
 - (A) ADDRESSEE: Genentech, Inc.
 - (B) STREET: 460 Point San Bruno Blvd
 - (C) CITY: South San Francisco
 - (D) STATE: California
 - (E) COUNTRY: USA
 - (F) ZIP: 94080
- 15 (v) COMPUTER READABLE FORM:
 - (A) MEDIUM TYPE: 3.5 inch, 1.44 Mb floppy disk
 - (B) COMPUTER: IBM PC compatible
 - (C) OPERATING SYSTEM: PC-DOS/MS-DOS
 - (D) SOFTWARE: WinPatin (Genentech)
- 20 (vi) CURRENT APPLICATION DATA:
 - (A) APPLICATION NUMBER:
 - (B) FILING DATE:
 - (C) CLASSIFICATION:
- 25 (vii) ATTORNEY/AGENT INFORMATION:
 - (A) NAME: Love, Richard B.
 - (B) REGISTRATION NUMBER: 34,659
 - (C) REFERENCE/DOCKET NUMBER: P0874P2PCT
- 30 (viii) TELECOMMUNICATION INFORMATION:
 - (A) TELEPHONE: 415/225-5530
 - (B) TELEFAX: 415/952-9881
 - (C) TELEX: 910/371-7168

(2) INFORMATION FOR SEQ ID NO:1:

- (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: Nucleic Acid
 - 35 (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- (xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

CAGTCCAACT GTTCAGGACG CC 22

(2) INFORMATION FOR SEQ ID NO:2:

- 40 (i) SEQUENCE CHARACTERISTICS:
 - (A) LENGTH: 22 base pairs
 - (B) TYPE: Nucleic Acid
 - (C) STRANDEDNESS: Single
 - (D) TOPOLOGY: Linear
- 45 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

GTGCTGCTCA TGCTGTAGGT GC 22

(2) INFORMATION FOR SEQ ID NO:3:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

GAAGTTGATG TCTTGTGAGT GGC 23

(2) INFORMATION FOR SEQ ID NO:4:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

GCATCCTAGA GTCACCGAGG AGCC 24

(2) INFORMATION FOR SEQ ID NO:5:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

CACTGGCTCA GGGAAATAAC CC 22

25 (2) INFORMATION FOR SEQ ID NO:6:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

GGAGAGCTGG GAAGGTGTGC AC 22

(2) INFORMATION FOR SEQ ID NO:7:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

40 ACAAACGCGT ACGCTGACAT CGTCATGACC CAGTC 35

(2) INFORMATION FOR SEQ ID NO:8:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

ACAAACGCGT ACGCTGATAT TGTCATGACT CAGTC 35

(2) INFORMATION FOR SEQ ID NO:9:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 35 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

ACAAACGCGT ACGCTGACAT CGTCATGACA CAGTC 35

(2) INFORMATION FOR SEQ ID NO:10:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 37 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

GCTCTTCGAA TGGTGGGAAG ATGGATACAG TTGGTGC 37

25 (2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
30 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

CGATGGGCC CGGATAGACCG ATGGGGCTGT TGTGTTGGC 39

(2) INFORMATION FOR SEQ ID NO:12:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

40 CGATGGGCC CGGATAGACTG ATGGGGCTGT CGTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:13:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

CGATGGGCC CGGATAGACGG ATGGGGCTGT TGTTTGCG 39

(2) INFORMATION FOR SEQ ID NO:14:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

CGATGGGCC CGGATAGACAG ATGGGGCTGT TGTTTGCG 39

(2) INFORMATION FOR SEQ ID NO:15:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

CGATGGGCC CGGATAGACCG ATGGGGCTGT TGTTTGCG 39

25 (2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
30 (C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

CGATGGGCC CGGATAGACTG ATGGGGCTGT TGTTTGCG 39

(2) INFORMATION FOR SEQ ID NO:17:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

40 CGATGGGCC CGGATAGACAG ATGGGGCTGT TGTTTGCG 39

(2) INFORMATION FOR SEQ ID NO:18:

5 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 39 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

CGATGGGCC CGATAGACGG ATGGGCTGT TGTGTTGGC 39

(2) INFORMATION FOR SEQ ID NO:19:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 369 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

GACATTGTCA TGACACAGTC TCAAAATTC ATGTCCACAT CAGTAGGAGA 50
 CAGGGTCAGC GTCACCTGCA AGGCCAGTCA GAATGTGGGT ACTAATGTAG 100
 CCTGGTATCA ACAGAAACCA GGGCAATCTC CTAAAGCACT GATTTACTCG 150
 TCATCCTACC GGTACAGTGG AGTCCCTGAT CGCTTCACAG GCAGTGGATC 200
 20 TGGGACAGAT TTCACTCTCA CCATCAGCCA TGTGCAGTCT GAAGACTTG 250
 CAGACTATT CTGTCAGCAA TATAACATCT ATCCTCTCAC GTTCGGTCCT 300
 GGGACCAAGC TGGAGTTGAA ACGGGCTGAT GCTGCACCAC CAACTGTATC 350
 CATCTTCCCA CCATTGAA 369

(2) INFORMATION FOR SEQ ID NO:20:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 123 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

30 Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val
 1 5 10 15
 Gly Asp Arg Val Ser Val Thr Cys Lys Ala Ser Gln Asn Val Gly
 20 25 30
 35 Thr Asn Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys
 35 40 45
 Ala Leu Ile Tyr Ser Ser Ser Tyr Arg Tyr Ser Gly Val Pro Asp
 50 55 60
 Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile
 65 70 75
 40 Ser His Val Gln Ser Glu Asp Leu Ala Asp Tyr Phe Cys Gln Gln

	80	85	90
	Tyr Asn Ile Tyr Pro Leu Thr Phe Gly Pro Gly Thr Lys Leu Glu		
	95	100	105
5	Leu Lys Arg Ala Asp Ala Ala Pro Pro Thr Val Ser Ile Phe Pro		
	110	115	120
	Pro Phe Glu		
	123		

(2) INFORMATION FOR SEQ ID NO:21:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 417 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

15 TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG 50
 GAGGCTTAGT GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAGCCTCT 100
 GGATTCAATAT TCAGTAGTTA TGGCATGTCT TGGGTTCGCC AGACTCCAGG 150
 CAAGAGCCTG GAGTTGGTCG CAACCATTAA TAATAATGGT GATAGCACCT 200
 ATTATCCAGA CAGTGTGAAG GGCGGATTCA CCATCTCCCG AGACAATGCC 250
 20 AAGAACACCCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC 300
 CATGTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT 350
 ACTGGGGCCA AGGGACTCTG GTCACTGTCT CTGCAGCCAA AACAAACAGCC 400
 CCATCTGTCT ATCCGGG 417

(2) INFORMATION FOR SEQ ID NO:22:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 130 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

30 Glu Val Gln Leu Val Glu Ser Gly Gly Gly Leu Val Pro Pro Gly
 1 5 10 15
 Gly Ser Leu Lys Leu Ser Cys Ala Ala Ser Gly Phe Ile Phe Ser
 20 25 30
 35 Ser Tyr Gly Met Ser Trp Val Arg Gln Thr Pro Gly Lys Ser Leu
 35 40 45
 Glu Leu Val Ala Thr Ile Asn Asn Asn Gly Asp Ser Thr Tyr Tyr
 50 55 60
 Pro Asp Ser Val Lys Gly Arg Phe Thr Ile Ser Arg Asp Asn Ala
 65 70 75
 40 Lys Asn Thr Leu Tyr Leu Gln Met Ser Ser Leu Lys Ser Glu Asp

80	85	90
Thr Ala Met Phe Tyr Cys Ala Arg Ala Leu Ile Ser Ser Ala Thr		
95 100 105		
Trp Phe Gly Tyr Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala		
5	110 115	120
Ala Lys Thr Thr Ala Pro Ser Val Tyr Pro		
125 130		

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

15 ACAAACGCGT ACGCTGATAT CGTCATGACA G 31

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 31 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

GCAGCATCAG CTCTTCGAAG CTCCAGCTTG G 31

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 21 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

CCACTAGTAC GCAAGTTCAC G 21

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 33 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Single
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

GATGGGCCCT TGGTGGAGGC TGCAGAGACA GTG 33

40 (2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 714 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 5 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50
 TGCTACAAAC GCGTACGCTG ATATCGTCAT GACACAGTCT CAAAAATTCA 100
 TGTCCACATC AGTAGGAGAC AGGGTCAGCG TCACCTGCAA GGCCAGTCAG 150
 10 AATGTGGGTA CTAATGTAGC CTGGTATCAA CAGAAACCAG GGCAATCTCC 200
 TAAAGCACTG ATTTACTCGT CATCCTACCG GTACAGTGGA GTCCCTGATC 250
 GCTTCACAGG CAGTGGATCT GGGACAGATT TCACTCTCAC CATCAGCCAT 300
 GTGCAGTCTG AAGACTTGGC AGACTATTTC TGTCAGCAAT ATAACATCTA 350
 TCCTCTCACG TTCGGTCCTG GGACCAAGCT GGAGCTTCGA AGAGCTGTGG 400
 15 CTGCACCACATC TGTCTTCATC TTCCCGCCAT CTGATGAGCA GTTGAAATCT 450
 GGAACTGCTT CTGTTGTGTG CCTGCTGAAT AACTTCTATC CCAGAGAGGC 500
 CAAAGTACAG TGGAAGGTGG ATAACGCCCT CCAATCGGGT AACTCCCAGG 550
 AGAGTGTACAG AGAGCAGGAC AGCAAGGACA GCACCTACAG CCTCAGCAGC 600
 ACCCTGACGC TGAGCAAAGC AGACTACGAG AACACAAAG TCTACGCCTG 650
 20 CGAAGTCACC CATCAGGGCC TGAGCTCGCC CGTCACAAAG AGCTTCAACA 700
 GGGGAGAGTG TTAA 714

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 237 amino acids
 25 (B) TYPE: Amino Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe
1														15

30	Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Asp	Ile	Val	Met	Thr	Gln	Ser
														30	

35	Gln	Lys	Phe	Met	Ser	Thr	Ser	Val	Gly	Asp	Arg	Val	Ser	Val	Thr
														45	

40	Cys	Lys	Ala	Ser	Gln	Asn	Val	Gly	Thr	Asn	Val	Ala	Trp	Tyr	Gln
														60	

45	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Ala	Leu	Ile	Tyr	Ser	Ser	Ser
														75	

50	Tyr	Arg	Tyr	Ser	Gly	Val	Pro	Asp	Arg	Phe	Thr	Gly	Ser	Gly	Ser
														90	

Gly Thr Asp Phe Thr Leu Thr Ile Ser His Val Gln Ser Glu Asp
 95 100 105
 Leu Ala Asp Tyr Phe Cys Gln Gln Tyr Asn Ile Tyr Pro Leu Thr
 110 115 120
 5 Phe Gly Pro Gly Thr Lys Leu Glu Leu Arg Arg Ala Val Ala Ala
 125 130 135
 Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys Ser
 140 145 150
 Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg
 10 155 160 165
 Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
 170 175 180
 Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
 185 190 195
 15 Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
 200 205 210
 Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser
 215 220 225
 Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
 20 230 235 237

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 756 base pairs
 (B) TYPE: Nucleic Acid
 25 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTCTAT 50
 TGCTACAAAC GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT 100
 30 TAGTGCCGCC TGGAGGGTCC CTGAAACTCT CCTGTGCAGC CTCTGGATTC 150
 ATATTCAAGTA GTTATGGCAT GTCTTGGGTT CGCCAGACTC CAGGCAAGAG 200
 CCTGGAGTTG GTCGCAACCA TTAATAATAA TGGTGATAGC ACCTATTATC 250
 CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAGAAC 300
 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT 350
 35 TTACTGTGCA AGAGCCCTCA TTAGTTCGGC TACTGGTTT GGTTACTGGG 400
 GCCAAGGGAC TCTGGTCACT GTCTCTGCAG CCTCCACCAA GGGCCCATCG 450
 GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC ACCTCTGGGG GCACAGCGGC 500
 CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG ACGGTGTCGT 550
 GGAACACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCCTA 600

CAGTCCTCAG GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG 650
 CAGCTTGGGC ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA 700
 ACACCAAGGT GGACAAGAAA GTTGAGCCA AATCTGTGA CAAAACTCAC 750
 ACATGA 756

5 (2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 251 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

10 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Glu Val Gln Leu Val Glu Ser
 20 25 30
 15 Gly Gly Gly Leu Val Pro Pro Gly Gly Ser Leu Lys Leu Ser Cys
 35 40 45
 Ala Ala Ser Gly Phe Ile Phe Ser Ser Tyr Gly Met Ser Trp Val
 50 55 60
 20 Arg Gln Thr Pro Gly Lys Ser Leu Glu Leu Val Ala Thr Ile Asn
 65 70 75
 Asn Asn Gly Asp Ser Thr Tyr Pro Asp Ser Val Lys Gly Arg
 80 85 90
 Phe Thr Ile Ser Arg Asp Asn Ala Lys Asn Thr Leu Tyr Leu Gln
 95 100 105
 25 Met Ser Ser Leu Lys Ser Glu Asp Thr Ala Met Phe Tyr Cys Ala
 110 115 120
 Arg Ala Leu Ile Ser Ser Ala Thr Trp Phe Gly Tyr Trp Gly Gln
 125 130 135
 30 Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser
 140 145 150
 Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr
 155 160 165
 Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
 170 175 180
 35 Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
 185 190 195
 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser
 200 205 210
 40 Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile
 215 220 225
 Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys
 230 235 240

Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
245 250 251

(2) INFORMATION FOR SEQ ID NO:31:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

10 CAGTCCAACT GTTCAGGACG CC 22

(2) INFORMATION FOR SEQ ID NO:32:

15 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

G TGCTGCTCA TGCTGTAGGT GC 22

(2) INFORMATION FOR SEQ ID NO:33:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 23 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

25 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

GAAGTTGATG TCTTGTGAGT GGC 23

(2) INFORMATION FOR SEQ ID NO:34:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 24 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

GCATCCTAGA GTCACCGAGG AGCC 24

35 (2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

CACTGGCTCA GGGAAATAAC CC 22

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 22 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

GGAGAGCTGG GAAGGGTGTGC AC 22

10 (2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

CCAATGCATA CGCTGACATC GTGATGACCC AGACCCCC 37

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

25 CCAATGCATA CGCTGATATT GTGATGACTC AGACTCC 37

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 37 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

CCAATGCATA CGCTGACATC GTGATGACAC AGACACC 37

(2) INFORMATION FOR SEQ ID NO:40:

35 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 35 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Single
- (D) TOPOLOGY: Linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

AGATGTCAAT TGCTCACTGG ATGGTGGGAA GATGG 35

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:

5 (A) LENGTH: 32 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

CAAACGCGTA CGCTGAGATC CAGCTGCAGC AG 32

10 (2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 32 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

CAAACGCGTA CGCTGAGATT CAGCTCCAGC AG 32

(2) INFORMATION FOR SEQ ID NO:43:

20 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

25 CGATGGGCC CGATAGACCG ATGGGGCTGT TGTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:

30 (A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

CGATGGGCC CGATAGACTG ATGGGGCTGT TGTTTGGC 39

(2) INFORMATION FOR SEQ ID NO:45:

35 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 39 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

40 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

CGATGGGCC CGATAGACAG ATGGGGCTGT TGTTTGGC 39

10 (2) INFORMATION FOR SEQ ID NO:46:

5 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 39 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

CGATGGGCC CGATAGACGG ATGGGGCTGT TGTTTGGC 39

10 (2) INFORMATION FOR SEQ ID NO:47:

15 (i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 391 base pairs
- (B) TYPE: Nucleic Acid
- (C) STRANDEDNESS: Double
- (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

GATATCGTGA TGACACAGAC ACCACTCTCC CTGCCTGTCA GTCTGGAGA 50

TCAGGCCTCC ATCTCTTGCA GATCTAGTCA GAGCCTTGTA CACGGTATTG 100

GAAACACCTA TTTACATTGG TACCTGCAGA AGCCAGGCCA GTCTCCAAAG 150

20 CTCCTGATCT ACAAAAGTTTC CAACCGATT TCTGGGGTCC CAGACAGGTT 200

CAGTGGCAGT GGATCAGGGA CAGATTCAC ACTCAGGATC AGCAGAGTGG 250

AGGCTGAGGA TCTGGGACTT TATTCTGCT CTCAAAGTAC ACATGTTCCG 300

CTCACGTTCG GTGCTGGAC CAAGCTGGAG CTGAAACGGG CTGATGCTGC 350

ACCAACTGTA TCCATCTTCC CACCATCCAG TGAGCAATTG A 391

25 (2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 131 amino acids
- (B) TYPE: Amino Acid
- (D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Asp	Ile	Val	Met	Thr	Gln	Thr	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu
1								10						15

Gly	Asp	Gln	Ala	Ser	Ile	Ser	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val
20									25					30

His	Gly	Ile	Gly	Asn	Thr	Tyr	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro
35								40						45

Gly	Gln	Ser	Pro	Lys	Leu	Leu	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe
50									55					60

Ser	Gly	Val	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp
65								70						75

Phe	Thr	Leu	Arg	Ile	Ser	Arg	Val	Glu	Ala	Glu	Asp	Leu	Gly	Leu	
80								85						90	
Tyr	Phe	Cys	Ser	Gln	Ser	Thr	His	Val	Pro	Leu	Thr	Phe	Gly	Ala	
95								100						105	
5	Gly	Thr	Lys	Leu	Glu	Leu	Lys	Arg	Ala	Asp	Ala	Ala	Pro	Thr	Val
				110					115						120
	Ser	Ile	Phe	Pro	Pro	Ser	Ser	Glu	Gln	Leu	Lys				
					125					130	131				

(2) INFORMATION FOR SEQ ID NO:49:

10 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 405 base pairs
 (B) TYPE: Nucleic Acid
 (C) STRANDEDNESS: Double
 (D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

GAGATTTCAGC	TGCAGCAGTC	TGGACCTGAG	CTGATGAAGC	CTGGGGCTTC	50
AGTGAAGATA	TCCTGCAAGG	CTTCTGGTTA	TTCATTCACT	AGCCACTACA	100
TGCAC TGGGT	GAAGCAGAGC	CATGGAAAGA	GCCTTGAGTG	GATTGGCTAC	150
ATTGATCCTT	CCAATGGTGA	AACTACTTAC	AACCAGAAAT	TCAAGGGCAA	200
20 GGCCACATTG	ACTGTAGACA	CATCTTCCAG	CACAGCCAAC	GTGCATCTCA	250
GCAGCCTGAC	ATCTGATGAC	TCTGCAGTCT	ATTTCTGTGC	AAGAGGGGAC	300
TATAGATACA	ACGGCAGTG	GT TTTTCGAT	GTCTGGGNG	NAGGGACCAC	350
GGTCACCGTC	TCCTCCGCCA	AAACCGACAG	CCCCATCGGT	CTATCCGGGC	400
CCATC	405				

25 (2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 135 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Glu	Ile	Gln	Leu	Gln	Gln	Ser	Gly	Pro	Glu	Leu	Met	Lys	Pro	Gly	
1				5					10					15	
Ala	Ser	Val	Lys	Ile	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Ser	Phe	Ser	
				20					25					30	
35	Ser	His	Tyr	Met	His	Trp	Val	Lys	Gln	Ser	His	Gly	Lys	Ser	Leu
				35					40					45	
	Glu	Trp	Ile	Gly	Tyr	Ile	Asp	Pro	Ser	Asn	Gly	Glu	Thr	Thr	Tyr
				50						55					60
40	Asn	Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Thr	Ser
				65					70					75	

Ser Ser Thr Ala Asn Val His Leu Ser Ser Leu Thr Ser Asp Asp
80 85 90
Ser Ala Val Tyr Phe Cys Ala Arg Gly Asp Tyr Arg Tyr Asn Gly
95 100 105
5 Asp Trp Phe Phe Asp Val Trp Gly Xaa Gly Thr Thr Val Thr Val
110 115 120
Ser Ser Ala Lys Thr Asp Ser Pro Ile Gly Leu Ser Gly Pro Ile
125 130 135

(2) INFORMATION FOR SEQ ID NO:51:

10 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO:51:

CTTGGTGGAG GCGGAGGAGA CG 22

(2) INFORMATION FOR SEQ ID NO:52:

20 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 38 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:52:

GAAACGGGCT GTTGCTGCAC CAACTGTATT CATCTTCC 38

25 (2) INFORMATION FOR SEQ ID NO:53:

30 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 31 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:53:

GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 31

(2) INFORMATION FOR SEQ ID NO:54:

35 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 22 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Single
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:54:

40 CTTGGTGGAG GCGGAGGAGA CG 22

(2) INFORMATION FOR SEQ ID NO:55:

5 (i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 729 base pairs
(B) TYPE: Nucleic Acid
(C) STRANDEDNESS: Double
(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:55:

ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTCTAT 50
TGCTACAAAT GCATACGCTG ATATCGTGAT GACACAGACA CCACCTCTCCC 100
TGCCTGTCAG TCTTGGAGAT CAGGCCTCCA TCTCTTGCAAG ATCTAGTCAG 150
10 AGCCTTGTAC ACGGTATTGG AAACACCTAT TTACATTGGT ACCTGCAGAA 200
GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTCC AACCGATTT 250
CTGGGGTCCC AGACAGGGTTC AGTGGCAGTG GATCAGGGAC AGATTCACA 300
CTCAGGATCA GCAGAGTGGA GGCTGAGGAT CTGGGACTTT ATTTCTGCTC 350
TCAAAGTACA CATGTTCCGC TCACGTTCGG TGCTGGGACC AAGCTGGAGC 400
15 TGAAACGGGC TGTTGCTGCA CCAACTGTAT TCATCTTCCC ACCATCCAGT 450
GAGCAATTGA AATCTGGAAC TGCCCTGTGTT GTGTGCCTGC TGAATAACTT 500
CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC GCCCTCCAAT 550
CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC 600
TACAGCCTCA GCAGCACCCCT GACGCTGAGC AAAGCAGACT ACGAGAAACA 650
20 CAAAGTCTAC GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA 700
CAAAGAGCTT CAACAGGGGA GAGTGTAA 729

(2) INFORMATION FOR SEQ ID NO:56:

25 (i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 242 amino acids
 (B) TYPE: Amino Acid
 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:56:

Met	Lys	Lys	Asn	Ile	Ala	Phe	Leu	Leu	Ala	Ser	Met	Phe	Val	Phe	
1				5					10				15		
30	Ser	Ile	Ala	Thr	Asn	Ala	Tyr	Ala	Asp	Ile	Val	Met	Thr	Gln	Thr
				20					25				30		
	Pro	Leu	Ser	Leu	Pro	Val	Ser	Leu	Gly	Asp	Gln	Ala	Ser	Ile	Ser
				35					40				45		
35	Cys	Arg	Ser	Ser	Gln	Ser	Leu	Val	His	Gly	Ile	Gly	Asn	Thr	Tyr
				50					55				60		
	Leu	His	Trp	Tyr	Leu	Gln	Lys	Pro	Gly	Gln	Ser	Pro	Lys	Leu	Leu
				65					70				75		
	Ile	Tyr	Lys	Val	Ser	Asn	Arg	Phe	Ser	Gly	Val	Pro	Asp	Arg	Phe
				80					85				90		

	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Arg	Ile	Ser	Arg
					95					100					105
	Val	Glu	Ala	Glu	Asp	Leu	Gly	Leu	Tyr	Phe	Cys	Ser	Gln	Ser	Thr
					110					115					120
5	His	Val	Pro	Leu	Thr	Phe	Gly	Ala	Gly	Thr	Lys	Leu	Glu	Leu	Lys
					125					130					135
	Arg	Ala	Val	Ala	Ala	Pro	Thr	Val	Phe	Ile	Phe	Pro	Pro	Ser	Ser
					140					145					150
10	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn
					155					160					165
	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn
					170					175					180
	Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp
					185					190					195
15	Ser	Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser
					200					205					210
	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr
					215					220					225
20	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly
					230					235					240
	Glu	Cys													
		242													

(2) INFORMATION FOR SEQ ID NO:57:

	(i) SEQUENCE CHARACTERISTICS:
25	(A) LENGTH: 762 base pairs
	(B) TYPE: Nucleic Acid
	(C) STRANDEDNESS: Double
	(D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:57:

30	ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT 50
	TGCTACAAAC GCGTACGCTG AGATTCAAGCT GCAGCAGTCT GGACCTGAGC 100
	TGATGAAGCC TGGGGCTTCA GTGAAGATAT CCTGCAAGGC TTCTGGTTAT 150
	TCATTCAGTA GCCACTACAT GCACTGGGTG AAGCAGAGCC ATGGAAAGAG 200
	CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA ACTACTTACA 250
35	ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC 300
	ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA 350
	TTTCTGTGCA AGAGGGACT ATAGATACAA CGGCGACTGG TTTTCGATG 400
	TCTGGGGCGC AGGGACCACG GTCACCGTCT CCTCCGCCTC CACCAAGGGC 450
	CCATCGGTCT TCCCCCTGGC ACCCTCCTCC AAGAGCACCT CTGGGGGCAC 500
40	AGCGGCCCTG GGCTGCCTGG TCAAGGACTA CTTCCCCGAA CCGGTGACGG 550

TGTCGTGGAA CTCAGGCGCC CTGACCAGCG GCGTGCACAC CTTCCCGGCT 600
 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC 650
 CTCCAGCAGC TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC 700
 CCAGCAACAC CAAGGTGGAC AAGAAAGTTG AGCCCAAATC TTGTGACAAA 750
 5 ACTCACACAT GA 762

(2) INFORMATION FOR SEQ ID NO:58:

(i) SEQUENCE CHARACTERISTICS:
 (A) LENGTH: 253 amino acids
 (B) TYPE: Amino Acid
 10 (D) TOPOLOGY: Linear

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:58:

Met Lys Lys Asn Ile Ala Phe Leu Leu Ala Ser Met Phe Val Phe
 1 5 10 15
 Ser Ile Ala Thr Asn Ala Tyr Ala Glu Ile Gln Leu Gln Gln Ser
 15 20 25 30
 Gly Pro Glu Leu Met Lys Pro Gly Ala Ser Val Lys Ile Ser Cys
 35 40 45
 Lys Ala Ser Gly Tyr Ser Phe Ser Ser His Tyr Met His Trp Val
 50 55 60
 20 Lys Gln Ser His Gly Lys Ser Leu Glu Trp Ile Gly Tyr Ile Asp
 65 70 75
 Pro Ser Asn Gly Glu Thr Thr Tyr Asn Gln Lys Phe Lys Gly Lys
 80 85 90
 Ala Thr Leu Thr Val Asp Thr Ser Ser Ser Thr Ala Asn Val His
 25 95 100 105
 Leu Ser Ser Leu Thr Ser Asp Asp Ser Ala Val Tyr Phe Cys Ala
 110 115 120
 Arg Gly Asp Tyr Arg Tyr Asn Gly Asp Trp Phe Phe Asp Val Trp
 125 130 135
 30 Gly Ala Gly Thr Thr Val Thr Val Ser Ser Ala Ser Thr Lys Gly
 140 145 150
 Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
 155 160 165
 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu
 35 170 175 180
 Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val
 185 190 195
 His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu
 200 205 210
 40 Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr
 215 220 225
 Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp

230

235

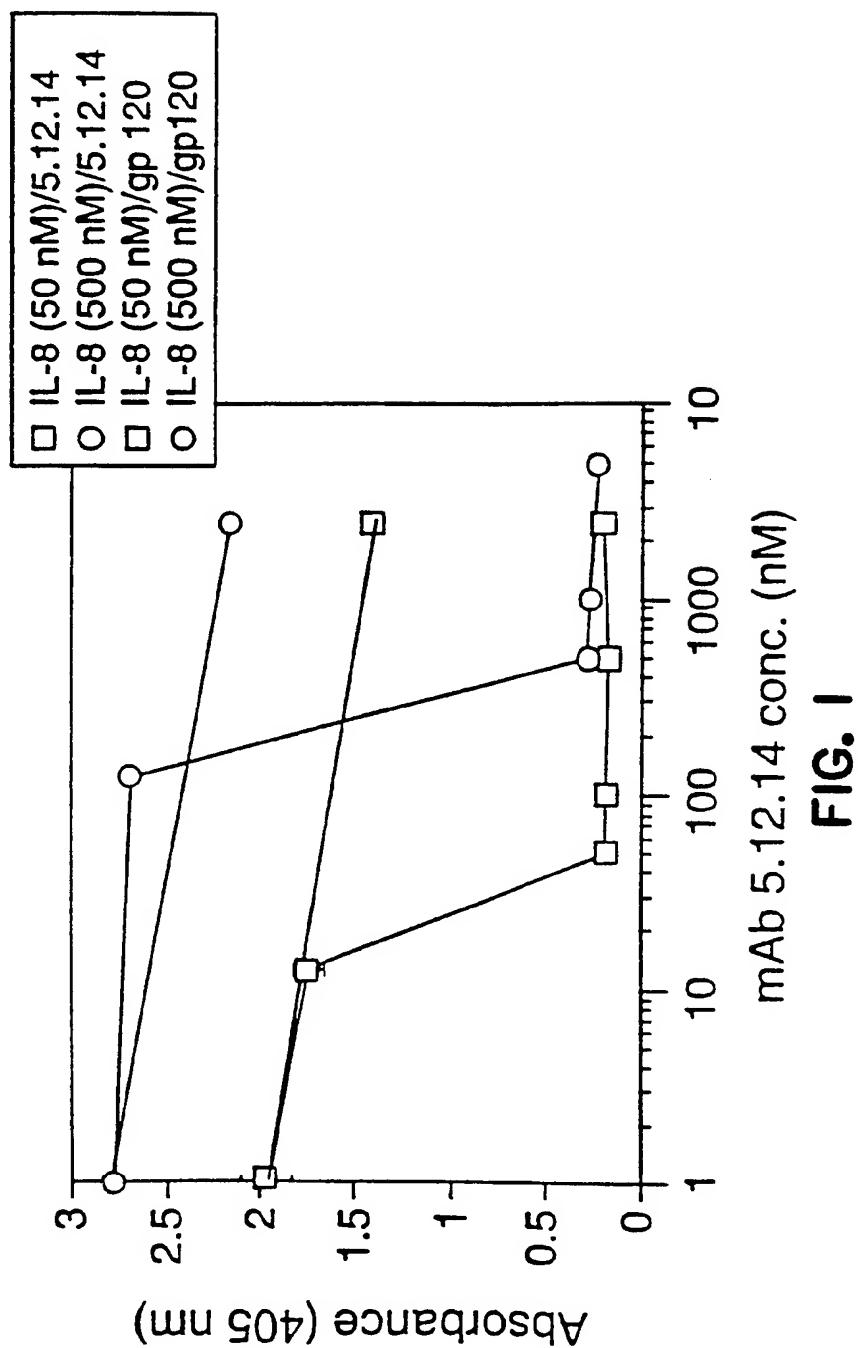
240

Lys Lys Val Glu Pro Lys Ser Cys Asp Lys Thr His Thr
245 250 253

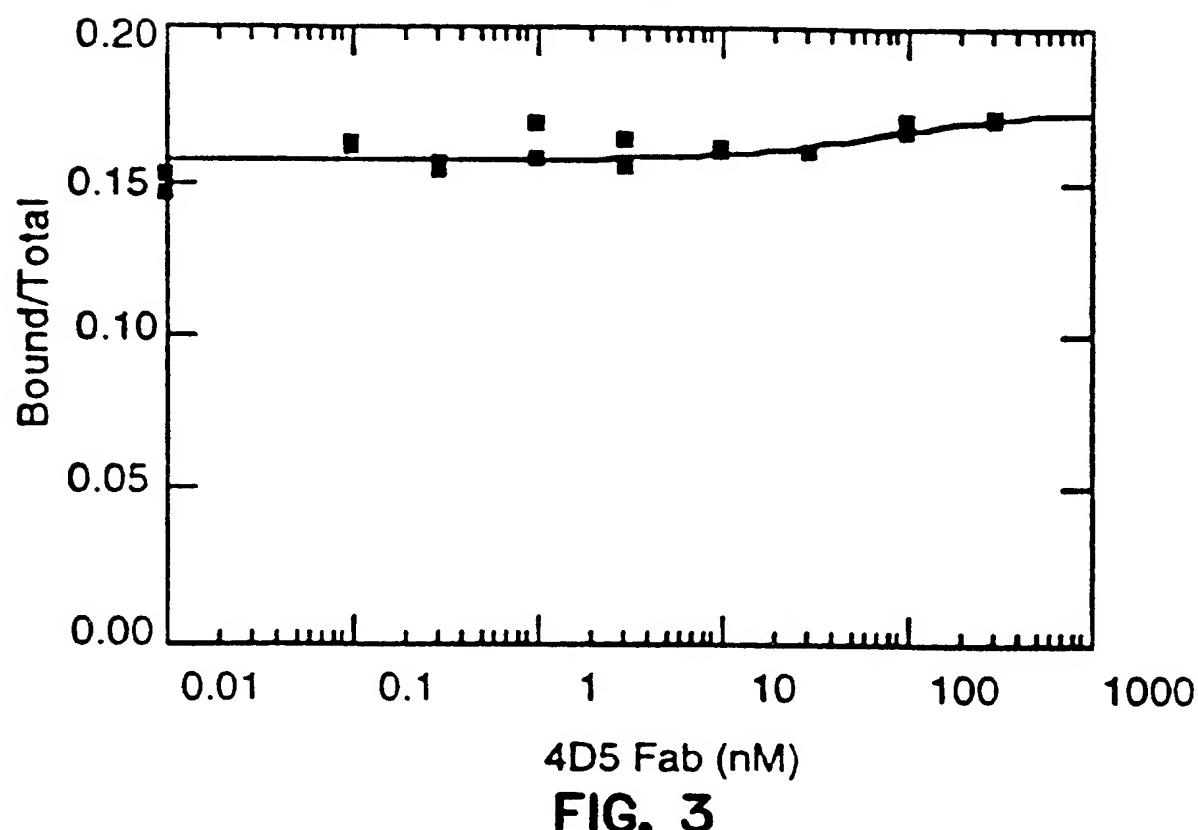
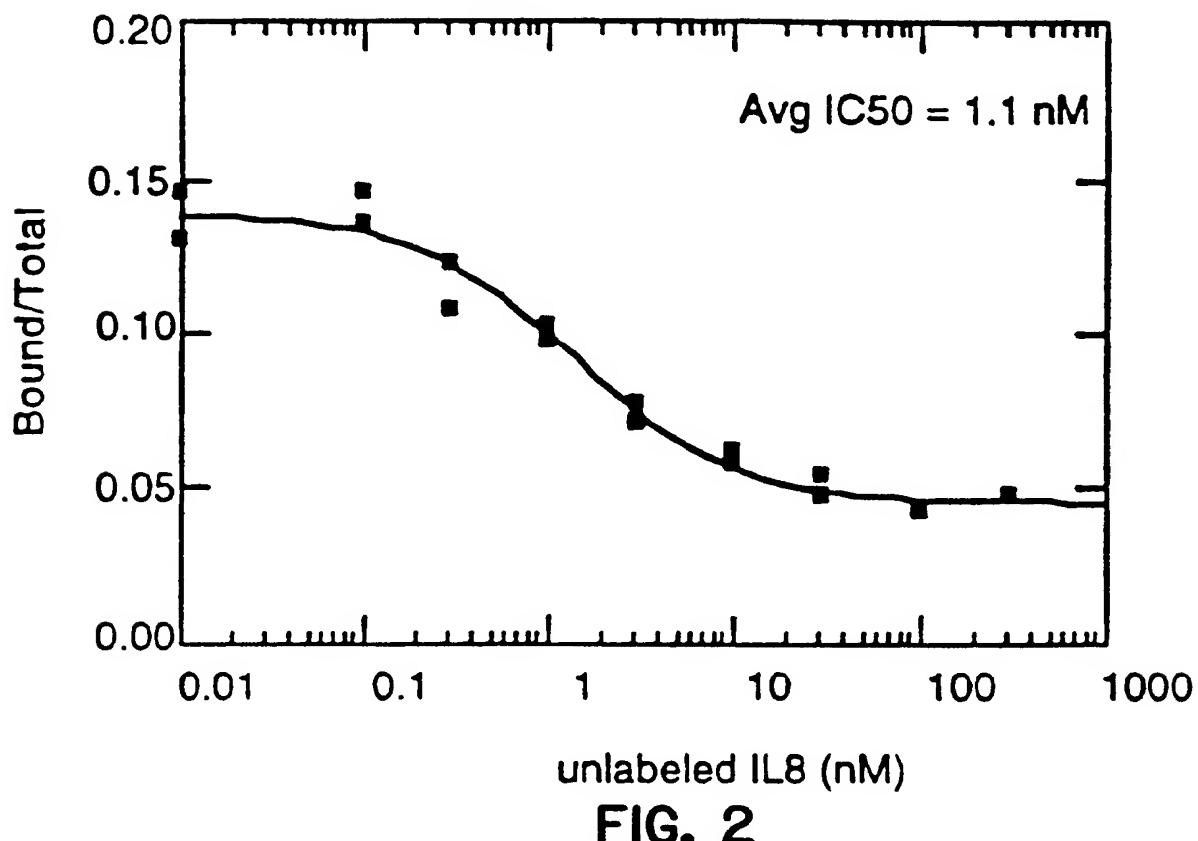
We Claim:

1. The use of an IL-8 antagonist in the manufacture of a medicament for treating asthma in a mammal.
2. The use of claim 1 wherein the asthma is allergic asthma.
- 5 3. The use of claim 1 wherein the mammal is a human.
4. The use of claim 1 wherein the IL-8 antagonist inhibits neutrophil chemotaxis in response to IL-8.
5. The use of claim 1 wherein the IL-8 antagonist inhibits IL-8 mediated elastase release by neutrophils.
- 10 6. The use of claim 1 wherein the IL-8 antagonist inhibits the binding of human IL-8 to human neutrophils.
7. The use of claim 1 wherein said medicament is administered to the mammal before the onset of asthma in the mammal.
- 15 8. The use of claim 1 wherein said medicament is administered to the mammal after the onset of asthma in the mammal.
9. The use of claim 1 wherein the IL-8 antagonist is an anti-IL-8 antibody.
10. The use of claim 9 wherein the anti-IL-8 antibody is a monoclonal antibody.
11. The use of claim 10 wherein the anti-IL-8 antibody is a chimeric antibody.
12. The use of claim 10 wherein the anti-IL-8 antibody is a humanized antibody.
- 20 13. The use of claim 10 wherein the anti-IL-8 antibody has the complementarity determining regions of 6G4.2.5.
14. The use of claim 10 wherein the anti-IL-8 antibody has the complementarity determining regions of 5.12.14.
- 25 15. A method for treating asthma in a mammal comprising administering an effective amount of an IL-8 antagonist to the mammal.

16. The method of claim 15 wherein the asthma is allergic asthma.
17. The method of claim 15 wherein the mammal is a human.
18. The method of claim 15 wherein the IL-8 antagonist inhibits neutrophil chemotaxis in response to IL-8.
- 5 19. The method of claim 15 wherein the IL-8 antagonist inhibits IL-8 mediated elastase release by neutrophils.
20. The method of claim 15 wherein the IL-8 antagonist inhibits the binding of human IL-8 to human neutrophils.
- 10 21. The method of claim 15 wherein the IL-8 antagonist is administered before the onset of asthma in the mammal.
22. The method of claim 15 wherein the IL-8 antagonist is administered after the onset of asthma in the mammal.
23. The method of claim 15 wherein the IL-8 antagonist is an anti-IL-8 antibody.
24. The method of claim 23 wherein the anti-IL-8 antibody is a monoclonal antibody.
- 15 25. The method of claim 24 wherein the anti-IL-8 antibody is a chimeric antibody.
26. The method of claim 25 wherein the anti-IL-8 antibody is a humanized antibody.
27. The method of claim 24 wherein the anti-IL-8 antibody has the complementarity determining regions of 6G4.2.5.
28. The method of claim 24 wherein the anti-IL-8 antibody has the complementarity determining regions of 5.12.14.

**FIG. 1**

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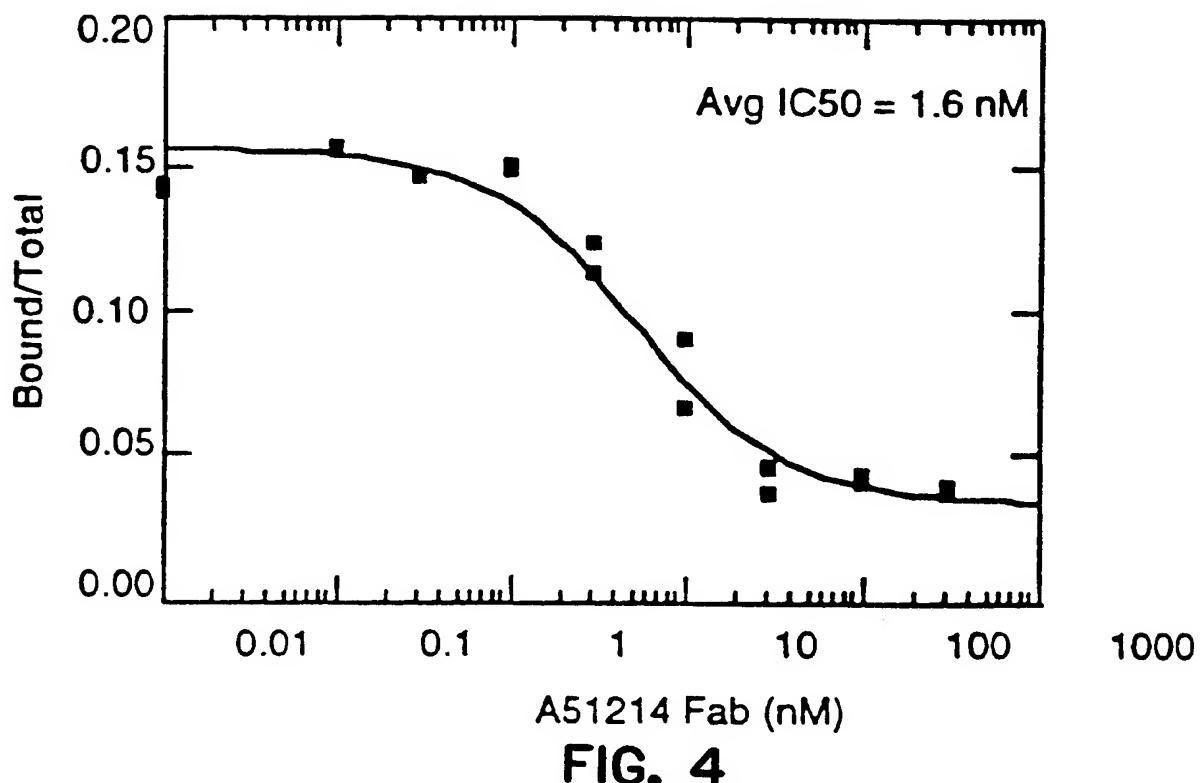


FIG. 4

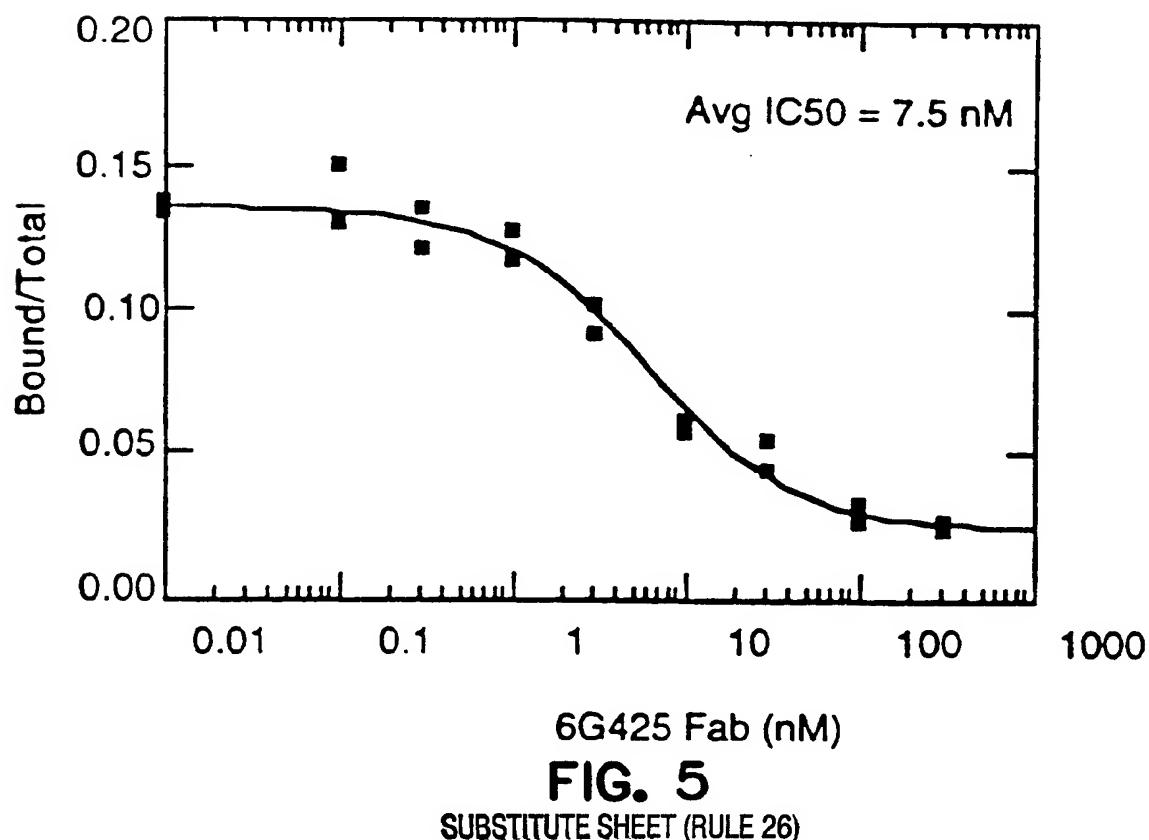


FIG. 5

SUBSTITUTE SHEET (RULE 26)

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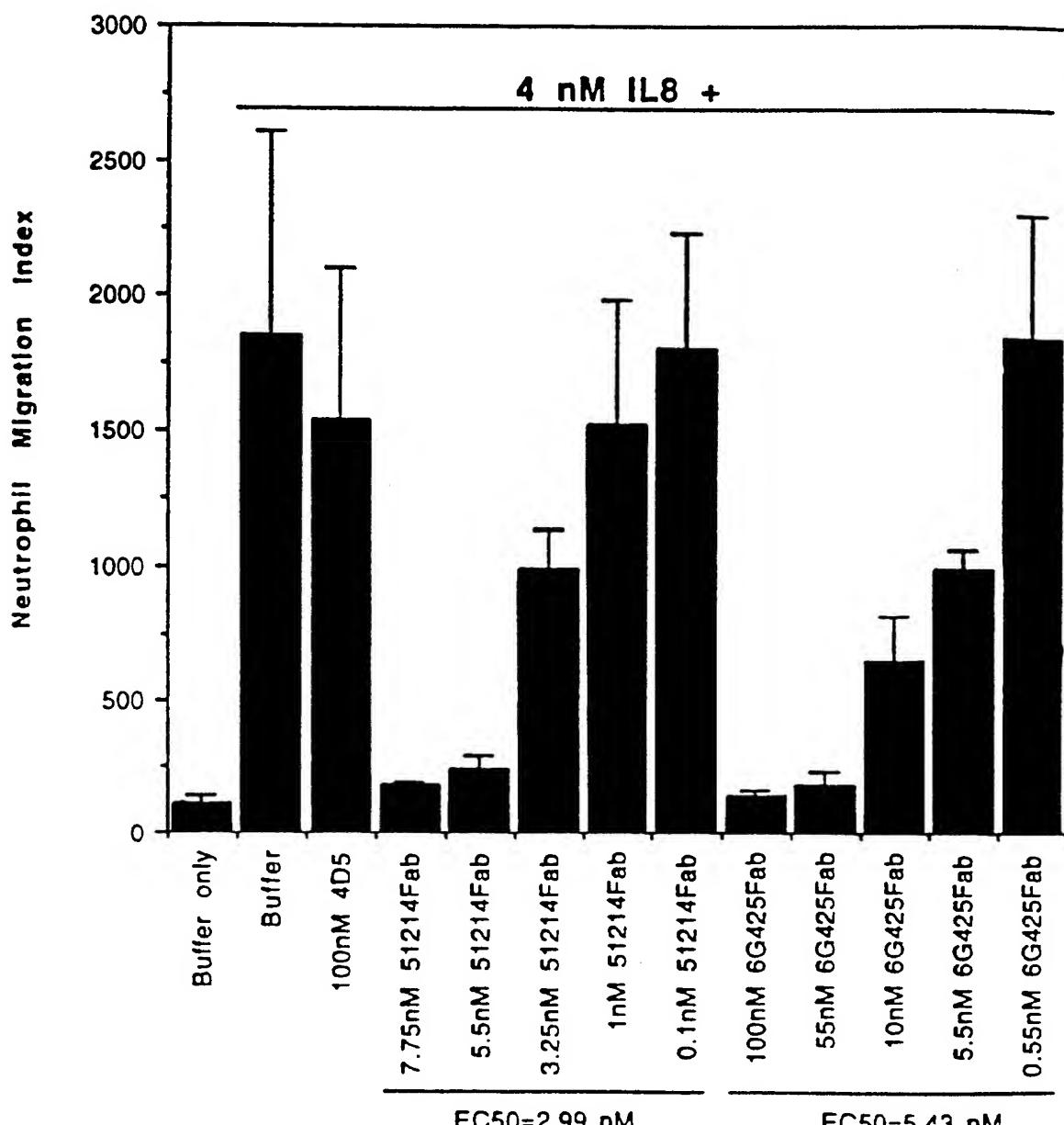


FIG. 6

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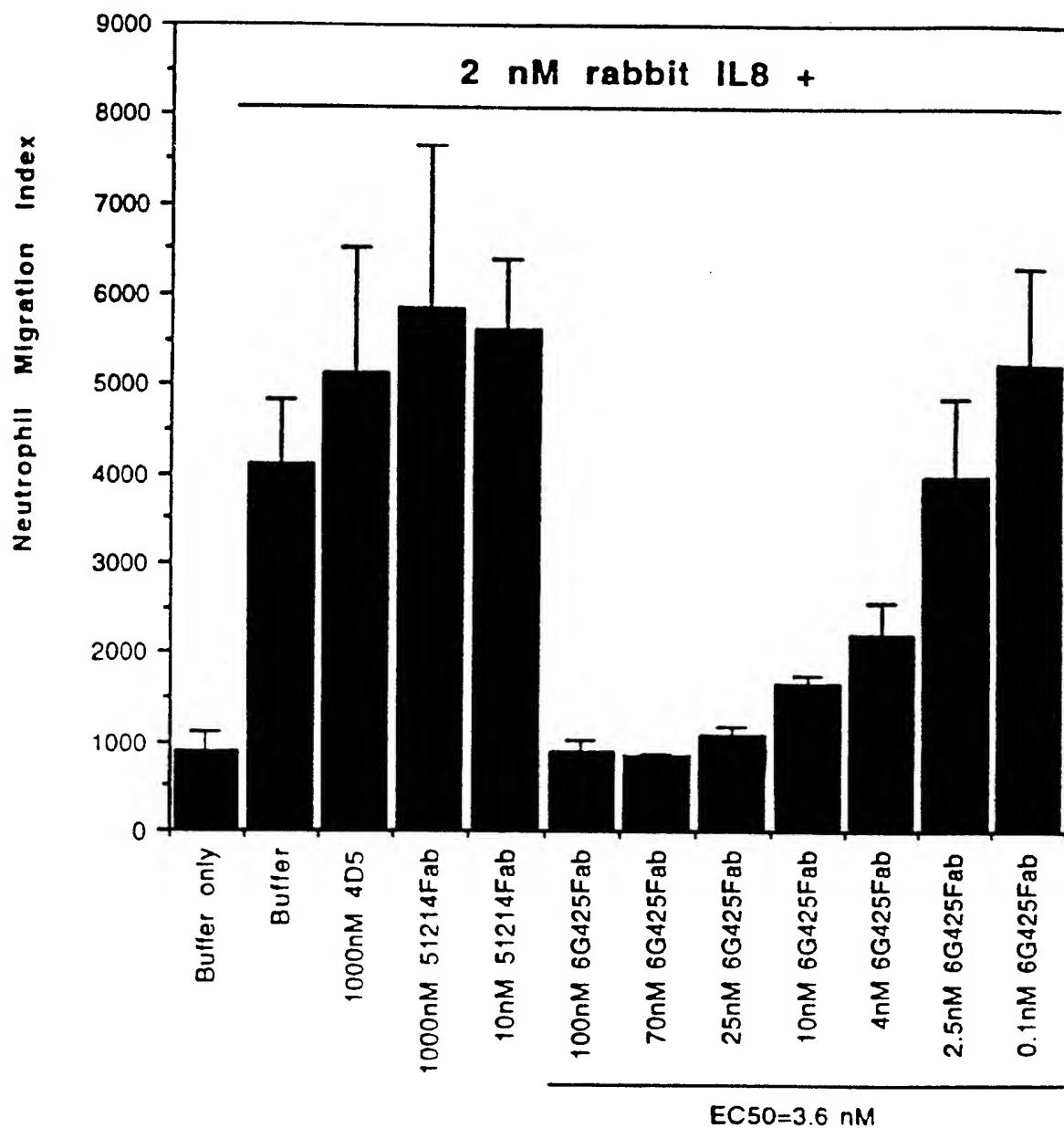
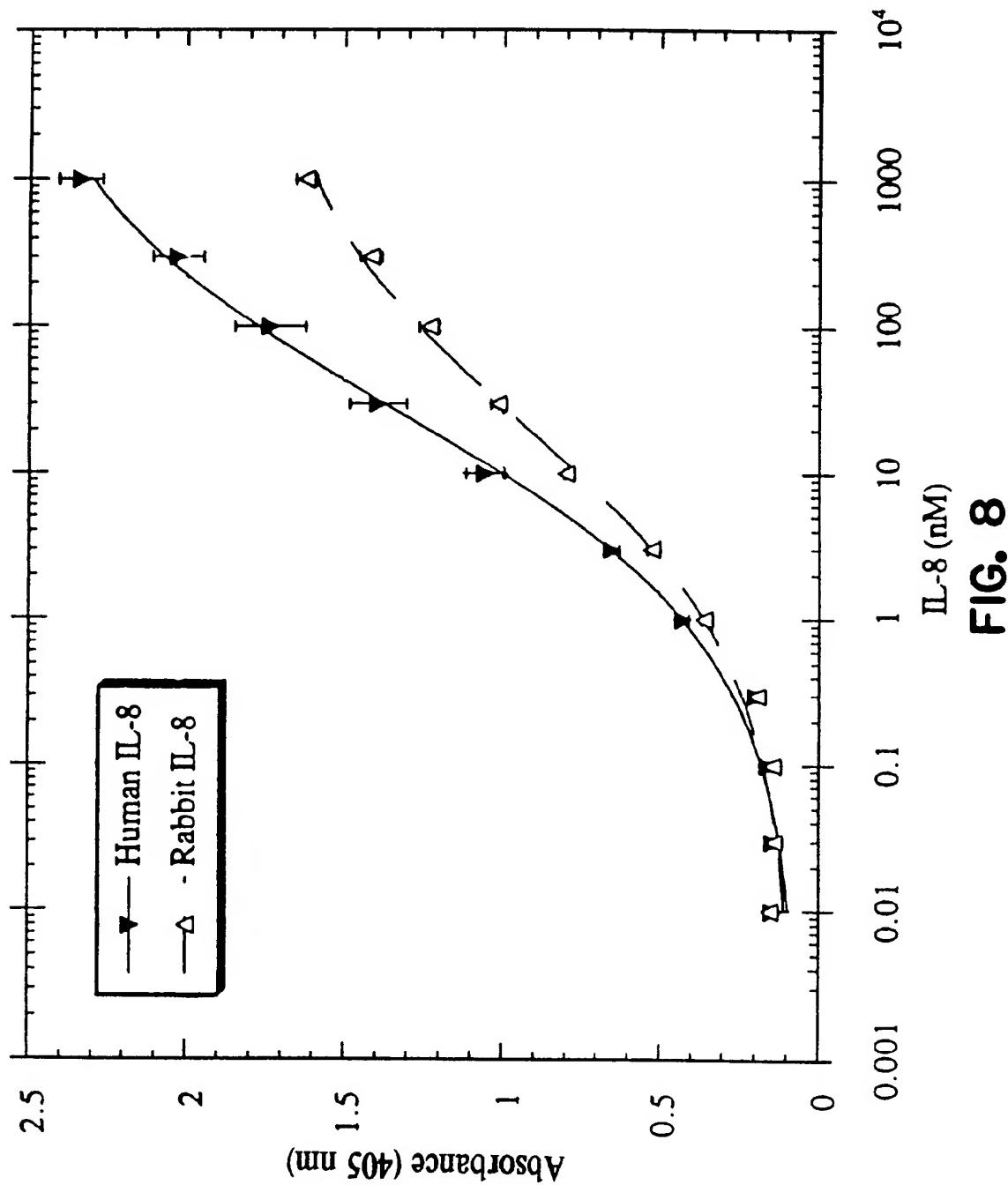
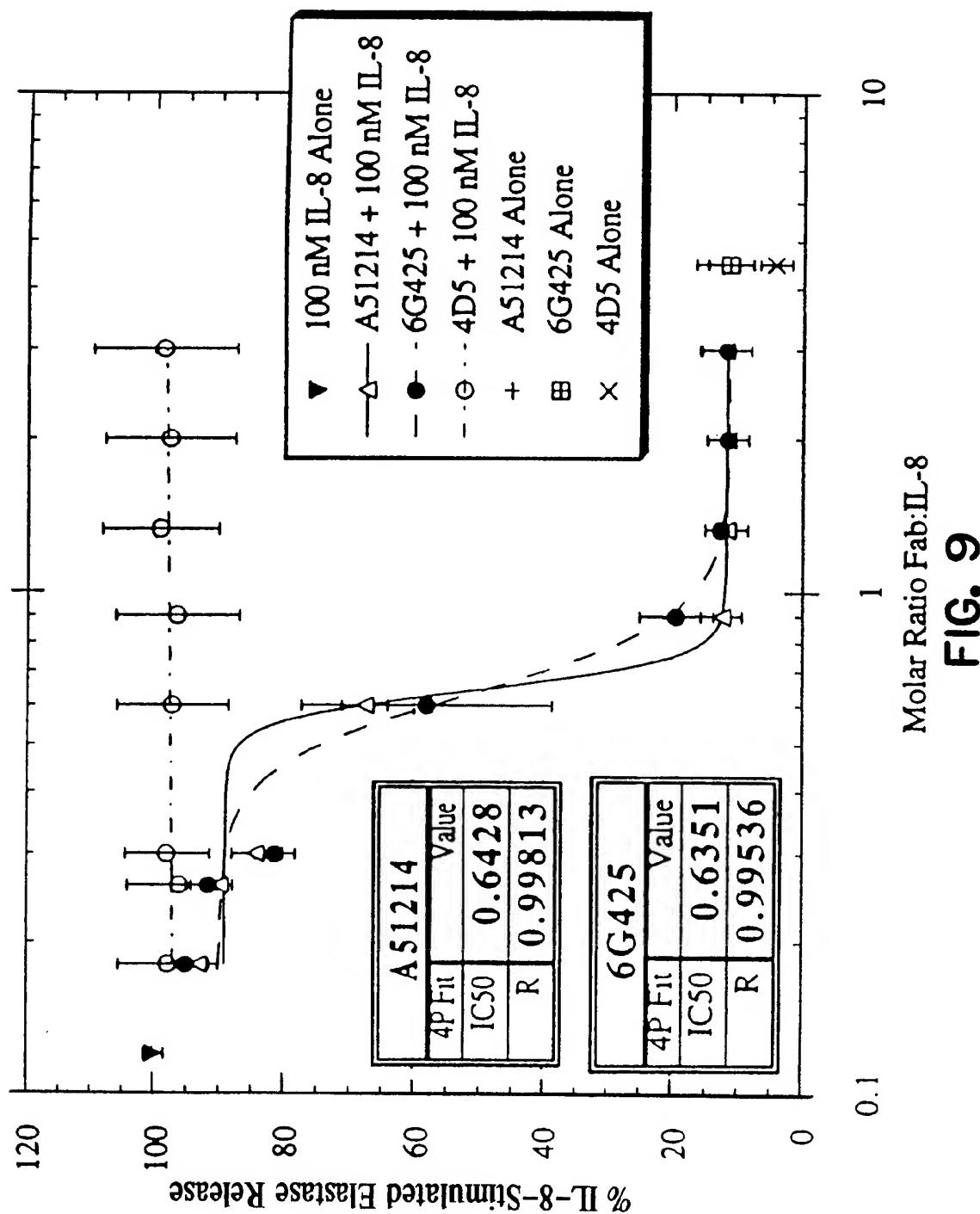
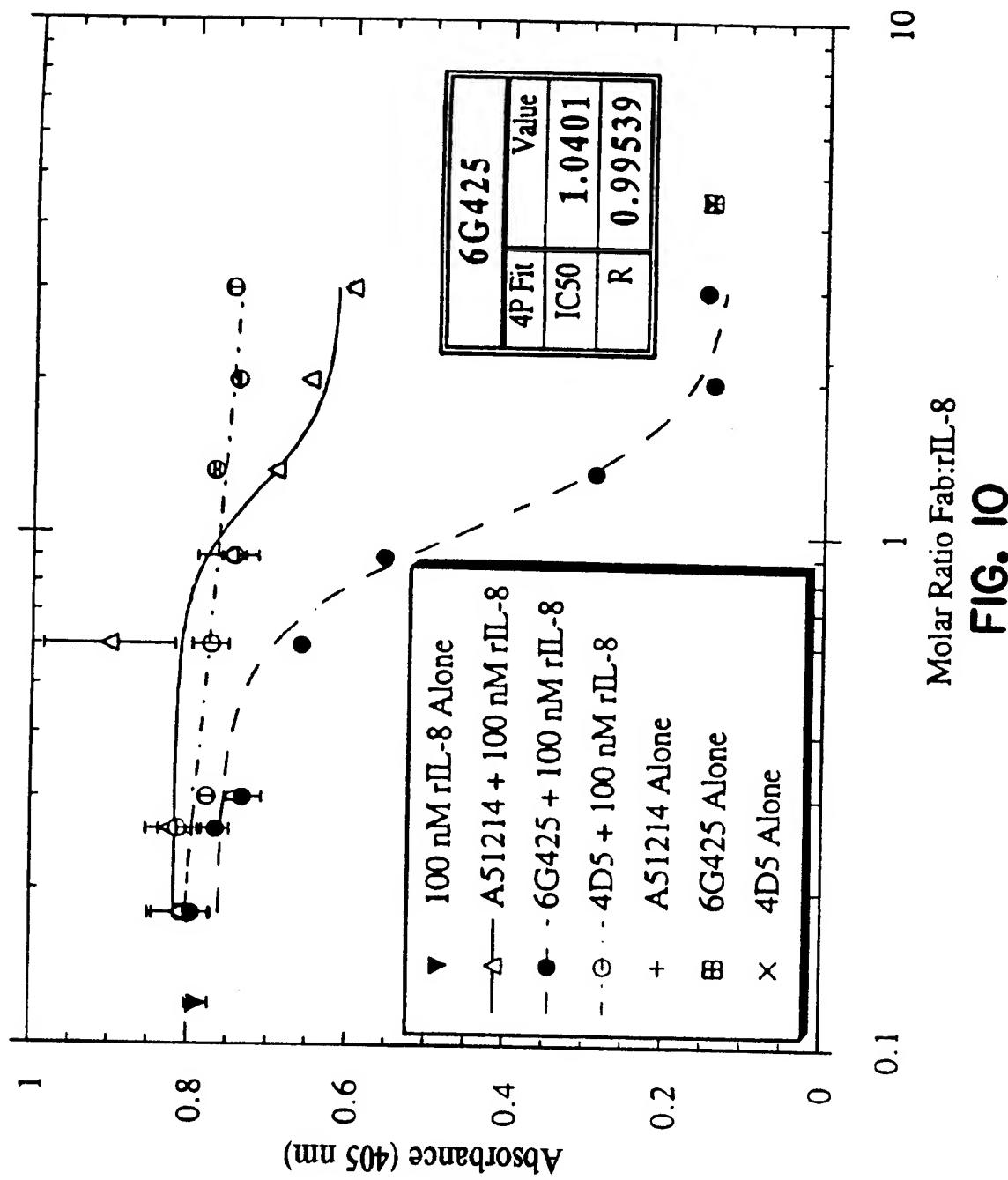


FIG. 7

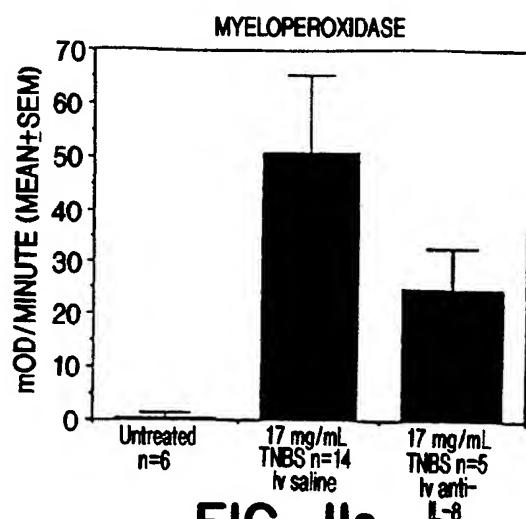
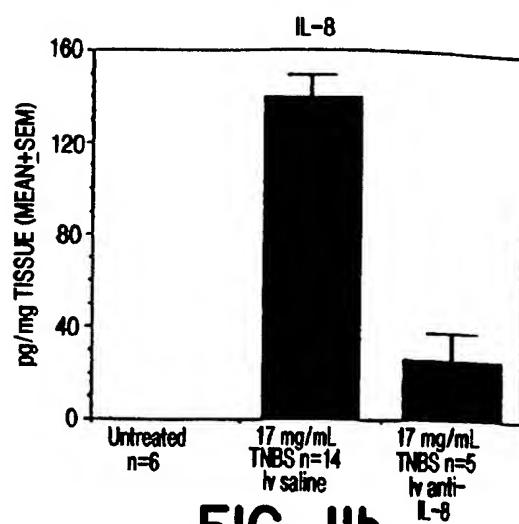
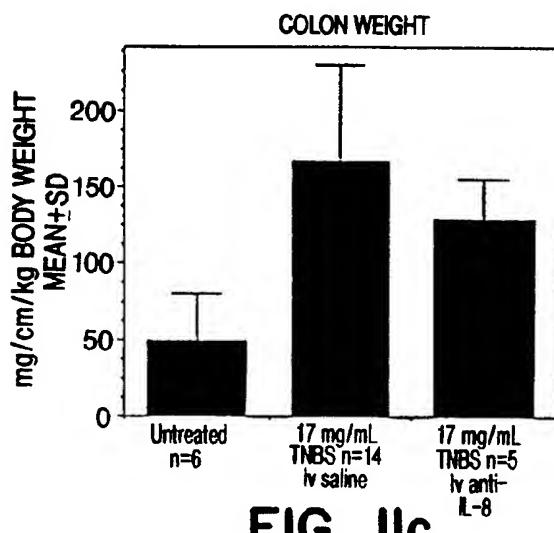
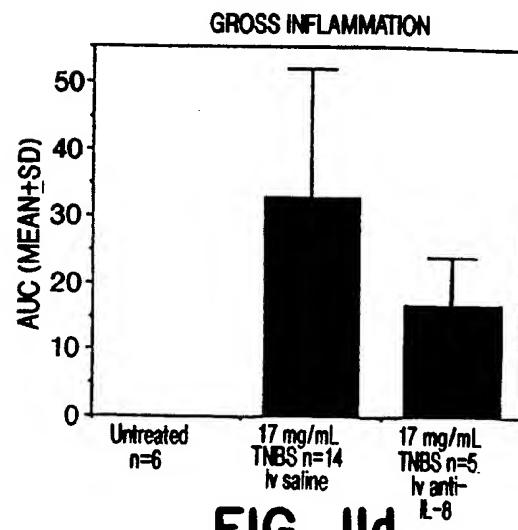
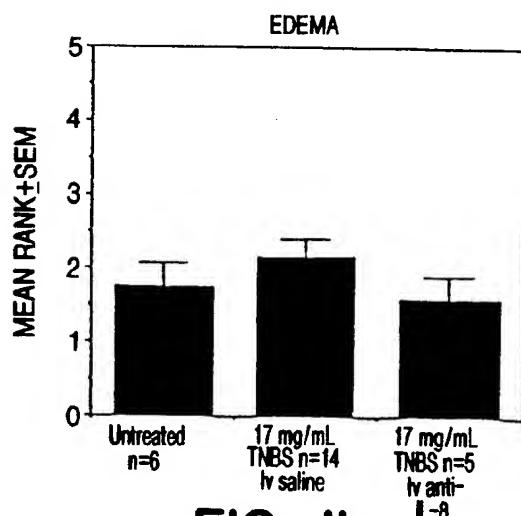
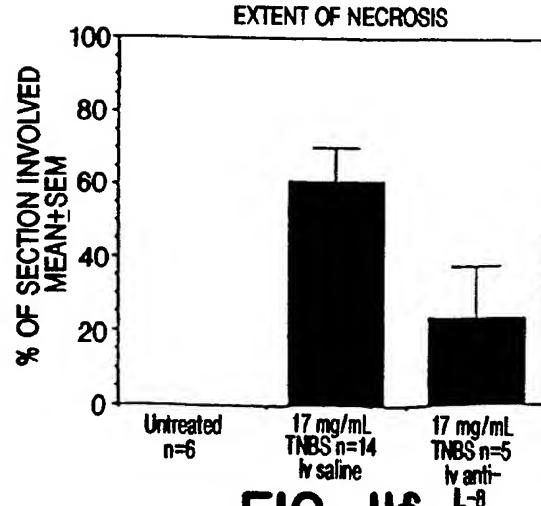
**FIG. 8**



1
Molar Ratio Fab:IL-8
FIG. 9



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**FIG. IIa****FIG. IIb****FIG. IIc****FIG. IId****FIG. IIe****FIG. IIff**

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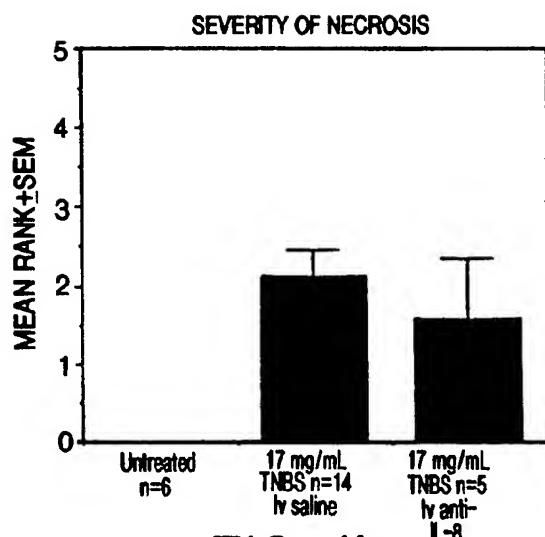


FIG. IIg

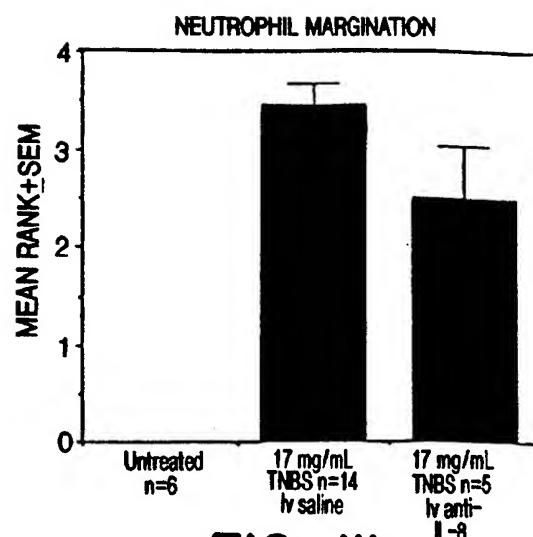


FIG. IIh

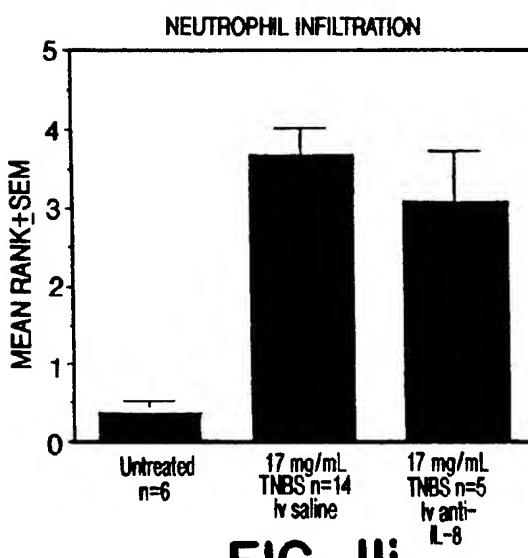


FIG. III

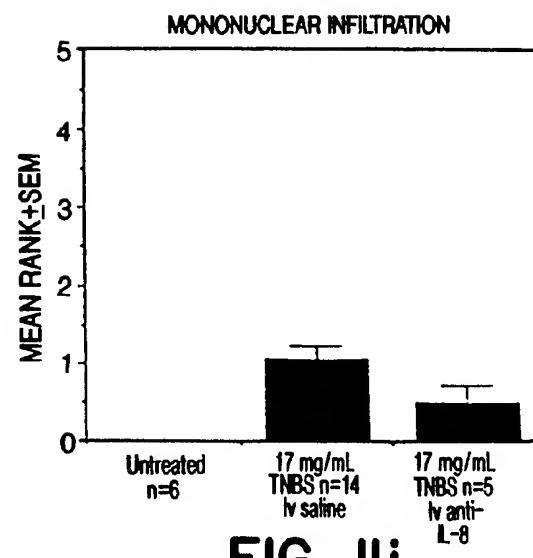


FIG. IIj

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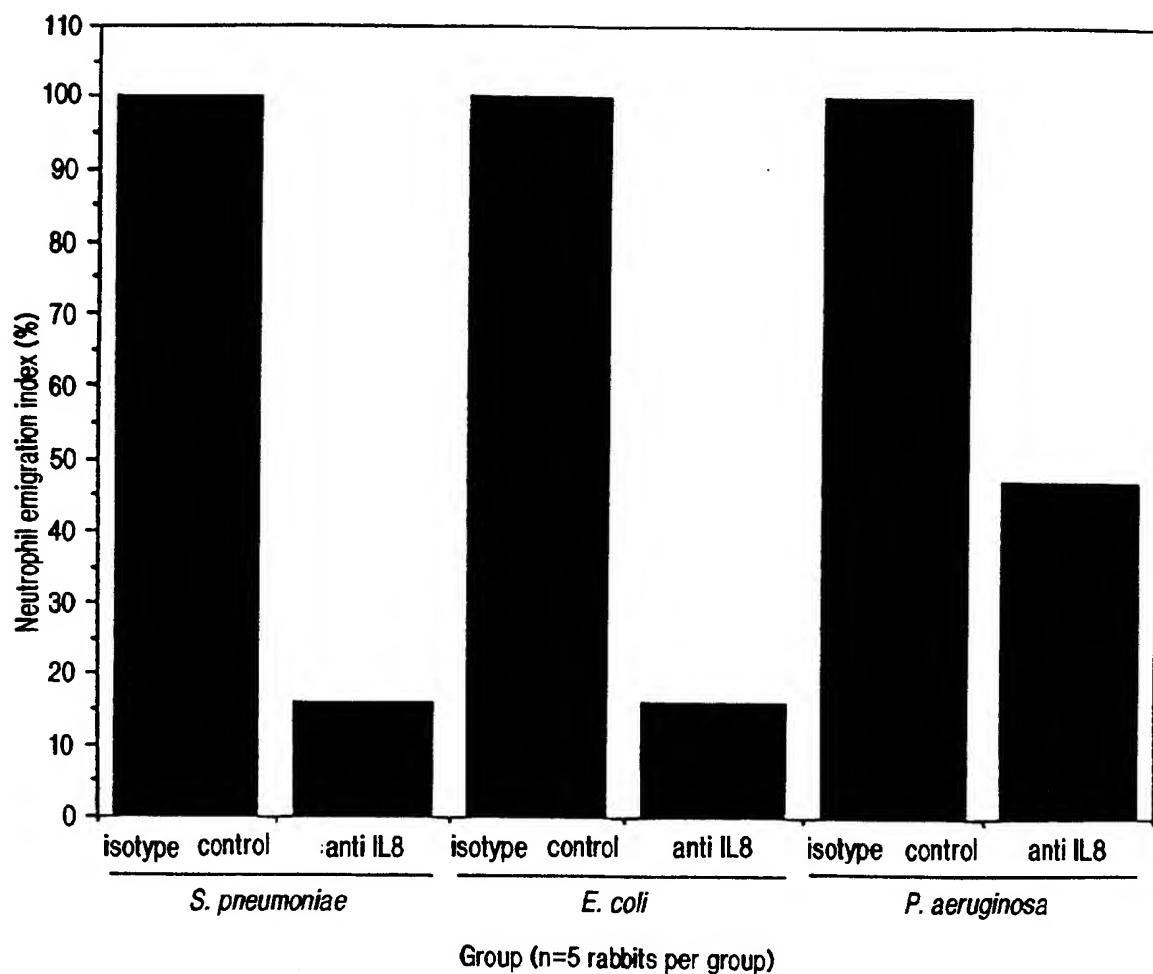


FIG. 12

Light Chain Primers:**MKLC-1, 22mer**

5' CAGTCCAACTGTTCAAGGACGCC 3' (SEQ ID NO:1)

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3' (SEQ ID NO:2)

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3' (SEQ ID NO:3)

Heavy Chain Primers:**IGG2AC-1, 24mer**

5' GCATCCTAGAGTCACCGAGGAGCC 3' (SEQ ID NO:4)

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3' (SEQ ID NO:5)

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3' (SEQ ID NO:6)

FIG. 13

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Light chain forward primer

SL001A-2 35 mer

5' ACAAACGCGTACGCT GACATCGTCATGACCCAGTC 3' (SEQ ID NO:7)
T T T (SEQ ID NO:8)
A (SEQ ID NO:9)

Light chain reverse primer

SL001B 37 mer

5' GCTCTTCGAATG GTGGGAAGATGGATACAGTTGGTGC 3' (SEQ ID NO:10)

FIG. 14

Heavy chain forward primer

SL002B 39 mer

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTGGC 3' (SEQ ID NO:11)
T C (SEQ ID NO:12)
G (SEQ ID NO:13)
A (SEQ ID NO:14)

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTGGC 3' (SEQ ID NO:15)
T (SEQ ID NO:16)
A (SEQ ID NO:17)
G (SEQ ID NO:18)

FIG. 15

Best BI

361 CCATTCGAA (SEQ ID NO:19)
GGTAAGCTT
121 P F E (SEQ ID NO:20)

FIG. 16

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1 TTCTATTGCT ACAAACGCGT ACGCTGAGGT GCAGCTGGTG GAGTCTGGGG GAGGCTTAGT
 AAGATAACGA TGTTTGCAGA TGCGACTCCA CGTCGACCCAC CTCAGACCCC CTCCGAATCA
 1 E V Q L V E S G G G L V

61 GCCGCCTGGA GGGTCCCTGA AACTCTCCTG TGCAAGCCTCT GGATTCATAT TCAGTAGTTA
 CGGCGGACCT CCCAGGGACT TTGAGAGGAC ACGTCGGAGA CCTAAGTATA AGTCATCAAT
 13 P P G G S L K L S C A A S G F I F S S Y

* * * * * CDR #1

121 TGGCATGTCT TGGGTTCGCC AGACTCCAGG CAAGAGCCTG GAGTTGGTCG CAACCATTAA
 ACCGTACAGA ACCCAAGCGG TCTGAGGTCC GTTCTCGGAC CTCAACCAGC GTTGGTAATT
 33 G M S W V R Q T P G K S L E L V A T I N
 * * * * *

181 TAATAATGGT GATAGCACCT ATTATCCAGA CAGTGTGAAG GGCCGATTCA CCATCTCCCG
 ATTATTACCA CTATCGTGGA TAATAGGTCT GTCAACACTTC CCGGCTAAGT GGTAGAGGGC
 53 N N G D S T Y Y P D S V K G R F T I S R
 * * * * *

* * * * * CDR #2

241 AGACAATGCC AAGAACACCC TGTACCTGCA AATGAGCAGT CTGAAGTCTG AGGACACAGC
 TCTGTTACGG TTCTTGTGGG ACATGGACGT TTACTCGTCA GACTTCAGAC TCCTGTGTCG
 73 D N A K N T L Y L Q M S S L K S E D T A

301 CATGTTTAC TGTGCAAGAG CCCTCATTAG TTCGGCTACT TGGTTTGGTT ACTGGGGCCA
 GTACAAAATG ACACGTTCTC GGGAGTAATC AAGCCGATGA ACCAAACCAA TGACCCCCGGT
 93 M F Y C A R A L I S S A T W F G Y W G Q

* * * * * * * * * * * CDR #3

361 AGGGACTCTG GTCACTGTCT CTGCAGCCAA AACAAACAGCC CCATCTGTCT
 TCCCTGAGAC CAGTGACAGA GACGTCGGTT TTGTTGTCGG GGTAGACAGA
 113 G T L V T V S A A K T T A P S V Y

Apal
 411 ATCCGGG (SEQ ID NO:21)
 TAGGCC
 130 P (SEQ ID NO:22)

FIG. 17

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VL.front 31-MER

5' ACAAACCGCGTACGCTGATATCGTCATGACAG 3' (SEQ ID NO:23)
VL.rear 31-MER5' GCAGCATCAGCTTTCGAAGCTCCAGCTTGG 3' (SEQ ID NO:24)

VH.front.SPE 21-MER

5' CCACTAGTACGCAAGTTCACG 3' (SEQ ID NO:25)

VH.rear 33-MER

5' GATGGGCCCTGGTGGAGGCTGCAGAGACAGTG 3' (SEQ ID NO:26)

FIG. 18

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FIG. 19
SUBSTITUTE SHEET (RULE 26)

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1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTCTAT TGCTACAAAC
 TACTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAAGC AAAAAAGATA ACCATGTTTG
 -23 M K K N I A F L L A S M F V F S I A T N

61 GCGTACGCTG AGGTGCAGCT GGTGGAGTCT GGGGGAGGCT TAGTGCCGCC TGGAGGGTCC
 CGCATGCGAC TCCACGTCGA CCACCTCAGA CCCCCCTCCGA ATCACGGCGG ACCTCCCAGG
 -3 A Y A E V Q L V E S G G G L V P P G G S

121 CTGAAACTCT CCTGTGCAGC CTCTGGATTC ATATTCACTA GTTATGGCAT GTCTGGGTT
 GACTTTGAGA GGACACGTCG GAGACCTAAG TATAAGTCAT CAATACCGTA CAGAACCCAA
 18 L K L S C A A S G F I F S S Y G M S W V

* * * * *

181 CGCCAGACTC CAGGCAAGAG CCTGGAGTGT GTCGCAACCA TTAATAATAA TGGTGATAGC
 GCGGTCTGAG GTCCGTTCTC GGACCTCAAC CAGCGTTGGT AATTATTATT ACCACTATCG
 38 R Q T P G K S L E L V A T I N N N G D S

* * * * *

241 ACCTATTATC CAGACAGTGT GAAGGGCCGA TTCACCATCT CCCGAGACAA TGCCAAAGAAC
 TGGATAATAG GTCTGTCACA CTTCCCGGT AAGTGGTAGA GGGCTCTGTT ACGGTTCTTG
 58 T Y Y P D S V K G R F T I S R D N A K N

* * * * *

CDR #2

301 ACCCTGTACC TGCAAATGAG CAGTCTGAAG TCTGAGGACA CAGCCATGTT TTACTGTGCA
 TGGGACATGG ACGTTACTC GTCAGACTTC AGACTCCTGT GTCGGTACAA AATGACACGT
 78 T L Y L Q M S S L K S E D T A M F Y C A

361 AGAGCCCTCA TTAGTCGGC TACTTGGTT GGTTACTGGG GCCAAGGGAC TCTGGTCACT
 TCTCGGGAGT AATCAAGCCG ATGAACCAAA CCAATGACCC CGGTTCCCTG AGACCAGTGA
 98 R A L I S S A T W F G Y W G Q G T L V T

* * * * *

CDR #3

ApaI

421 GTCTCTGCAG CCTCCACCAA GGGCCCATCG GTCTTCCCCC TGGCACCCCTC CTCCAAGAGC
 CAGAGACGTC GGAGGTGGTT CCCGGGTAGC CAGAAGGGGG ACCGTGGGAG GAGGTTCTCG
 118 V S A A S T K G P S V F P L A P S S K S

481 ACCTCTGGGG GCACAGCGGC CCTGGGCTGC CTGGTCAAGG ACTACTTCCC CGAACCGGTG
 TGGAGACCCC CGTGTGCGCCG GGACCCGACG GACCAGTTCC TGATGAAGGG GCTTGGCCAC
 138 T S G G T A A L G C L V K D Y F P E P V

541 ACGGTGTCGT GGAACTCAGG CGCCCTGACC AGCGGCGTGC ACACCTTCCC GGCTGTCTA
 TGCCACAGCA CCTTGAGTCC GCGGGACTGG TCGCCCGACG TGTGGAAGGG CCGACAGGAT
 158 T V S W N S G A L T S G V H T F P A V L

601 CAGTCCTCA GACTCTACTC CCTCAGCAGC GTGGTGACCG TGCCCTCCAG CAGCTTGGGC
 GTCAGGAGTC CTGAGATGAG GGAGTCGTG CACCACTGGC ACGGGAGGTC GTCGAACCCG
 178 Q S S G L Y S L S S V V T V P S S S L G

661 ACCCAGACCT ACATCTGCAA CGTGAATCAC AAGCCCAGCA ACACCAAGGT GGACAAGAAA
 TGGGTCTGGA TGTAGACGTT GCACCTAGTG TTCGGGTGCT TGTGGTTCCA CCTGTTCTTT
 198 T Q T Y I C N V N H K P S N T K V D K K

721 GTTGAGCCCA AATCTTGTGA CAAAACTCAC ACATGGA (SEQ ID NO:29)
 CAACTCGGGT TTAGAACACT GTTTTGAGTG TGTACT

218 V E P K S C D K T H T O (SEQ ID NO:30)

FIG. 20
SUBSTITUTE SHEET (RULE 26)

Light Chain Primers:**MKLC-1, 22mer**

5' CAGTCCAACTGTTCAGGACGCC 3' (SEQ ID NO:31)

MKLC-2, 22mer

5' GTGCTGCTCATGCTGTAGGTGC 3' (SEQ ID NO:32)

MKLC-3, 23mer

5' GAAGTTGATGTCTTGTGAGTGGC 3' (SEQ ID NO:33)

Heavy Chain Primers:**IGG2AC-1, 24mer**

5' GCATCCTAGAGTCACCGAGGAGCC 3' (SEQ ID NO:34)

IGG2AC-2, 22mer

5' CACTGGCTCAGGGAAATAACCC 3' (SEQ ID NO:35)

IGG2AC-3, 22mer

5' GGAGAGCTGGGAAGGTGTGCAC 3' (SEQ ID NO:36)

FIG. 2I

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Light chain forward primer

6G4.light.Nsi 36-MER

5' CCAATGCATACGCT GAC ATC GTG ATG ACC CAG ACC CC 3' (SEQ ID NO:37)
T T T T (SEQ ID NO:38)
A A (SEQ ID NO:39)

Light chain reverse primer

6G4.light.Mun 35-MER

5' AGA TGT CAA TTG CTC ACT GGA TGG TGG GAA GAT GG 3' (SEQ ID NO:40)

FIG. 22

Heavy chain forward primer

6G4.heavy.Mlu 32-MER

5' CAAACGCGTACGCT GAG ATC CAG CTG CAG CAG 3' (SEQ ID NO:41)
T C (SEQ ID NO:42)

Heavy chain reverse primer

SL002B 39-MER

5' CGATGGGCCCGG ATAGACCGATGGGGCTGTTGTTGGC 3' (SEQ ID NO:43)
T (SEQ ID NO:44)
A (SEQ ID NO:45)
G (SEQ ID NO:46)

FIG. 23

FIG. 24

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FIG. 25

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5' CTTGGTGGAGGC GGAGGAGACG 3' (SEQ ID NO:51)

Mutagenesis Primer for 6G425VL

DS/VF 38MER

5' GAAACGGGCTGTTGCTGCACCAACTGTATT CATCTTCC 3' (SEQ ID NO:52)

SYN.BstEII 31 MER

5' GTCACCGTCT CCTCCGCCTC CACCAAGGGC C 3' (SEQ ID NO:53)

SYN.Apa 22 MER

5' CTTGGTGGAGGC GGAGGAGACG 3' (SEQ ID NO:54)

FIG. 26

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1 ATGAAGAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTCTAT TGCTACAAAT
TACTTCTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTA
-23 M K K N I A F L L A S M F V F S I A T N

61 GCATACGCTG ATATCGTGAT GACACAGACA CCACTCTCCC TGCCCTGTCAAG TCTTGGAGAT
 CGTATGCGAC TATAGCACTA CTGTGTCTGT GGTGAGAGGG ACGGACAGTC AGAACCTCTA
 -3 A Y A D I V M T Q T P L S L P V S L G D

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121 CAGGCTCCA TCTCTTGCAG ATCTAGTCAG AGCCTTGTAC ACGGTATTGG AAACACCTAT
GTCCGGAGGT AGAGAACGTC TAGATCAGTC TCGAACATG TGCCATAACC TTTGTGGATA
 18 Q A S I S C R S S O S L V H G I G N T Y

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CDR #1

181 TTACATTGGT ACCTGCAGAA GCCAGGCCAG TCTCCAAAGC TCCTGATCTA CAAAGTTTCC
 AATGTAACCA TGGACGTCTT CGGTCCGGTC AGAGGTTTCG AGGACTAGAT GTTCAAAAGG
 38 L H W Y L Q K P G O S P K L L I Y K V S

CDR #2

241 AACCGATTTT CTGGGGTCCC AGACAGGTTG AGTGGCAGTG GATCAGGGAC AGATTTACAA
 TTGGCTAAAA GACCCCAGGG TCTGTCCAAG TCACCGTCAC CTAGTCCCTG TCTAAAGTGT
 58 N R F S G V P D R F S G S G S G T D E T

20

301 CTCAGGATCA GCAGAGTGGG CGCTGAGGAT CTGGGACTTT ATTTCTGCTC TCAAAAGTACA
 GAGTCCTAGT CGTCTCACCT CCGACTCCCTA GACCCCTGAAA TAAAGACGGAG AGTTTCATGT
 78 L R I S R V E A E D L G L Y F C S O S T

CDR #3

361 CATGTTCCGC TCACGGTCGG TGCTGGGACCC AAGCTGGAGC TGAAACGGGC TGTGCTGCA
 GTACAAAGGCG AGTGCAAGCC ACGACCCCTGG TTTCGACCTCG ACTTTGCCCG ACAACGACGT
 96 H V P L T F G A G T K L E L K R A V A A

| | | | | | | | | | | | | | | | | | | | | |
|-----|------------|------------|------------|------------|------------|-------------|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 421 | CCAACTGTAT | TCATCTTCCC | ACCATCCAGT | GAGCAATTGA | AATCTGGAAC | TGCTCTGTGTT | | | | | | | | | | | | | | |
| | GTTGACATA | AGTAGAAGGG | TGGTAGCTCA | CTCGTTAACT | TTAGACCTTG | ACGGAGACAA | | | | | | | | | | | | | | |
| 118 | P | T | V | F | I | F | P | P | S | S | E | Q | L | K | S | G | T | A | S | V |

481 GTGTGCCTGC TGAATAACCTT CTATCCCAGA GAGGCCAAAG TACAGTGGAA GGTGGATAAC
 CACACGGGACG ACTTATTGAA GATAGGGTCT CTCCGGTTTC ATGTCACCTT CCACCTATTG
 138 V C L L N N F Y P R E A K V Q W K V D N

541 GCCCTCCAAT CGGGTAACTC CCAGGAGAGT GTCACAGAGC AGGACAGCAA GGACAGCACC
 CGGGAGGTTA GCCCATTGAG GGTCTCTCA CAGTGTCTCG TCCTGTCTGG CCTGTCTGG
 158 A L Q S G N S Q E S V T E Q D S K D S T

601 TACAGCCTCA GCAGCACCCCT GAGCCTGAGC AAAGCAGACT ACGAGAAACA CAAAGTCTAC
 ATGTCGGAGT CGTCGTGGGA CTGCGACTCG TTTCGTCTGA TGCTCTTGT GTTTCAGATG
 178 Y S L S S T L T L S R A D Y E K H K V Y

661 GCCTGCGAAG TCACCCATCA GGGCCTGAGC TCGCCCGTCA CAAAGAGCTT CAACAGGGGA
 CGGACGCTTC AGTGGGTAGT CCCGGACTCG AGCGGGCAGT GTTTCTCGAA GTTGTCCCCCT
 198 A C E V T H Q G L S S P V T K S F N R G

721 GAGTGTAA (SEQ ID NO:55)
CTCACAAATT
218 E C O (SEQ ID NO:56)

FIG. 27

SUBSTITUTE SHEET (RULE 26)

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1 ATGAAAAAGA ATATCGCATT TCTTCTTGCA TCTATGTTCG TTTTTTCTAT TGCTACAAAC
 TACTTTTCT TATAGCGTAA AGAAGAACGT AGATACAAGC AAAAAAGATA ACGATGTTTG
 -23 M K K N I A F L L A S M F V F S I A T N

61 GCGTACGCTG AGATTCAGCT GCAGCAGTCT GGACCTGAGC TGATGAAGCC TGGGGCTTCA
 CGCATGCGAC TCTAAGTCGA CGTCGTCAGA CCTGGACTCG ACTACTTCGG ACCCCGAAGT
 -3 A Y A E I Q L Q Q S G P E L M K P G A S

121 GTGAAGATAT CCTGCAAGGC TTCTGGTTAT TCATTCAGTA GCCACTACAT GCACTGGGTG
 CACTTCTATA GGACGTTCCG AAGACCAATA AGTAAGTCAT CGGTGATGTA CGTGACCCAC
 18 V K I S C K A S G Y S F S S H Y M H W V

* * * * *

CDR #1

181 AAGCAGAGCC ATGGAAAGAG CCTTGAGTGG ATTGGCTACA TTGATCCTTC CAATGGTGAA
 TTCGTCTCGG TACCTTTCTC GGAACTCACC TAACCGATGT AACTAGGAAG GTTACCACTT
 38 K Q S H G K S L E W I G Y I D P S N G E

* * * * *

CDR #2

241 ACTACTTACA ACCAGAAATT CAAGGGCAAG GCCACATTGA CTGTAGACAC ATCTTCCAGC
 TGATGAATGT TGGTCTTAA GTTCCCGTTC CGGTGTAAC GACATCTGTG TAGAAGGTG
 58 T T Y N Q K F K G K A T L T V D T S S S

301 ACAGCCAACG TGCATCTCAG CAGCCTGACA TCTGATGACT CTGCAGTCTA TTTCTGTGCA
 TGTCGGTTGC ACGTAGAGTC GTCGGACTGT AGACTACTGA GACGTCAGAT AAAGACACGT
 78 T A N V H L S S L T S D D S A V Y F C A

361 AGAGGGGACT ATAGATACAA CGGCGACTGG TTTTTCGATG TCTGGGGCGC AGGGACCACG
 TCTCCCTGTA TATCTATGTT GCCGCTGACC AAAAGCTAC AGACCCCGCG TCCCTGGTGC
 98 R G D Y R Y N G D W F F D V W G A G T T

* * * * *

CDR #3

421 GTCACCGTCT CCTCCGCCTC CACCAAGGGC CCATCGGTCT TCCCCCTGGC ACCCTCCTCC
 CAGTGGCAGA GGAGGCGGAG GTGGTCTCCG GGTAGCCAGA AGGGGGACCG TGGGAGGAGG
 118 V T V S S A S T K G P S V F P L A P S S

481 AAGAGCACCT CTGGGGGCAC AGCGGCCCTG GGCTGCTGG TCAAGGACTA CTTCCCCGAA
 TTCTCGTGGA GACCCCCGTG TCGCCGGGAC CCGACGGACC AGTTCTGTAT GAAGGGGCTT
 138 K S T S G G T A A L G C L V K D Y F P E

541 CCGGTGACGG TGTCGTGGAA CTCAGGGGCC CTGACCGAGG GCGTGACAC CTTCCCGGCT
 GCCCACTGCC ACAGCACCTT GAGTCCGGCG GACTGGTCGC CGCACGTGTG GAAGGGCCGA
 158 P V T V S W N S G A L T S G V H T F P A

601 GTCCTACAGT CCTCAGGACT CTACTCCCTC AGCAGCGTGG TGACCGTGCC CTCCAGCAGC
 CAGGATGTCA GGAGTCTGA GATGAGGGAG TCGTCGCACC ACTGGCACCG GAGGTCGTG
 178 V L Q S S G L Y S L S S V V T V P S S S

661 TTGGGCACCC AGACCTACAT CTGCAACGTG AATCACAAGC CCAGCAACAC CAAGGTGGAC
 AACCCGTGGG TCTGGATGTA GACGTTGCAC TTAGTGTTCG GGTCGTTGTG GTTCCACCTG
 198 L G T Q T Y I C N V N H K P S N T K V D

721 AAGAAAGTTG AGCCCAAATC TTGTGACAAA ACTCACACAT GA (SEQ ID NO:57)
 TTCTTTCAAC TCGGGTTTAG AACACTGTT TGAGTGTGTA CT
 218 K K V E P K S C D K T H T O (SEQ ID NO:58)

FIG. 28
SUBSTITUTE SHEET (RULE 26)

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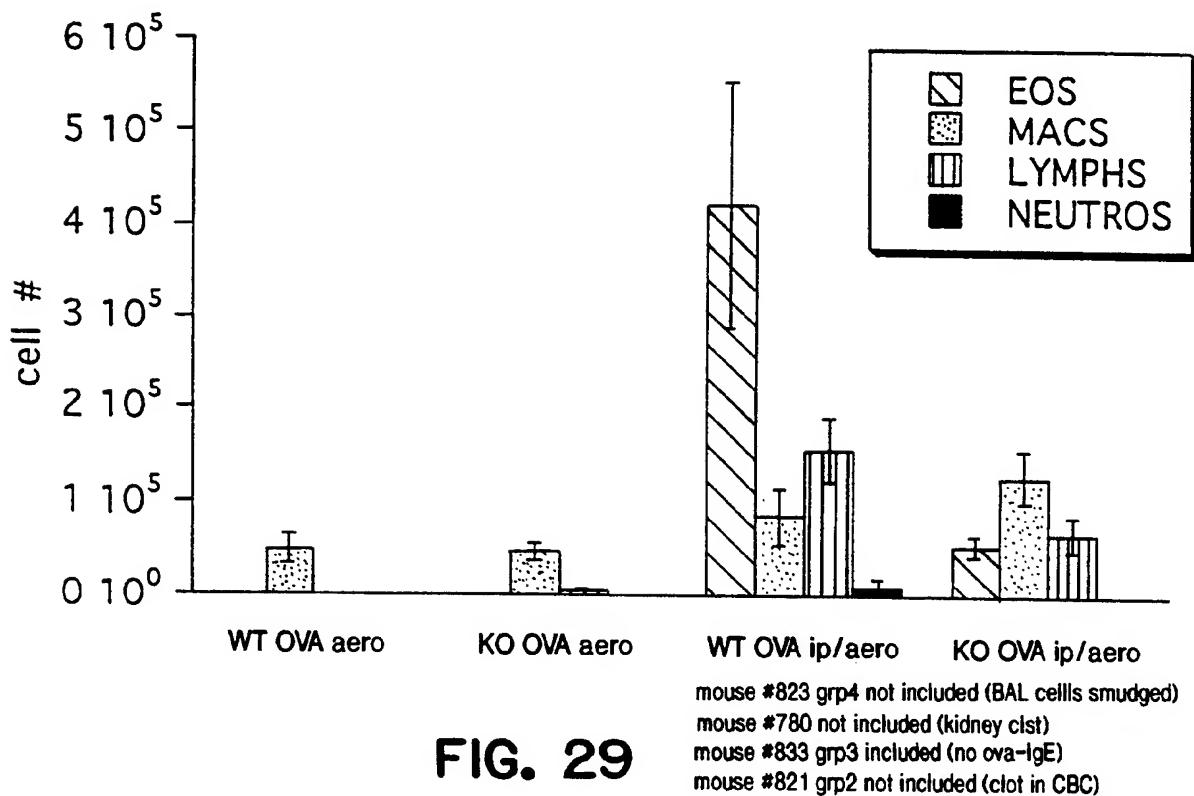


FIG. 29

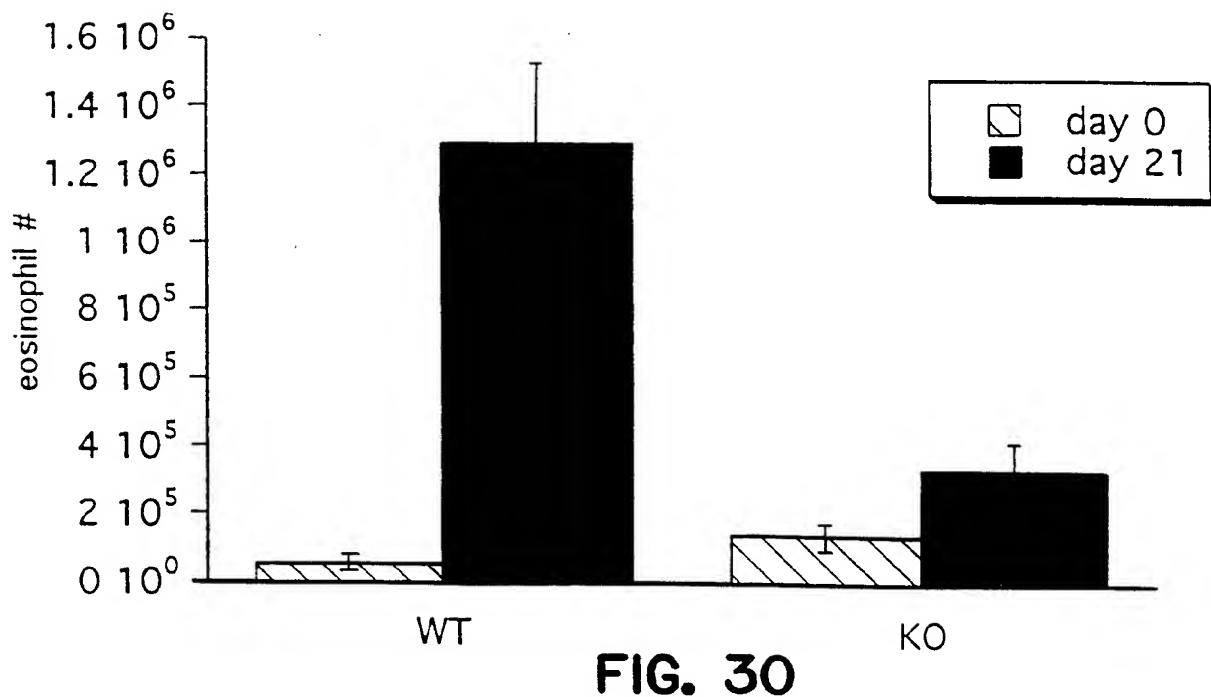


FIG. 30

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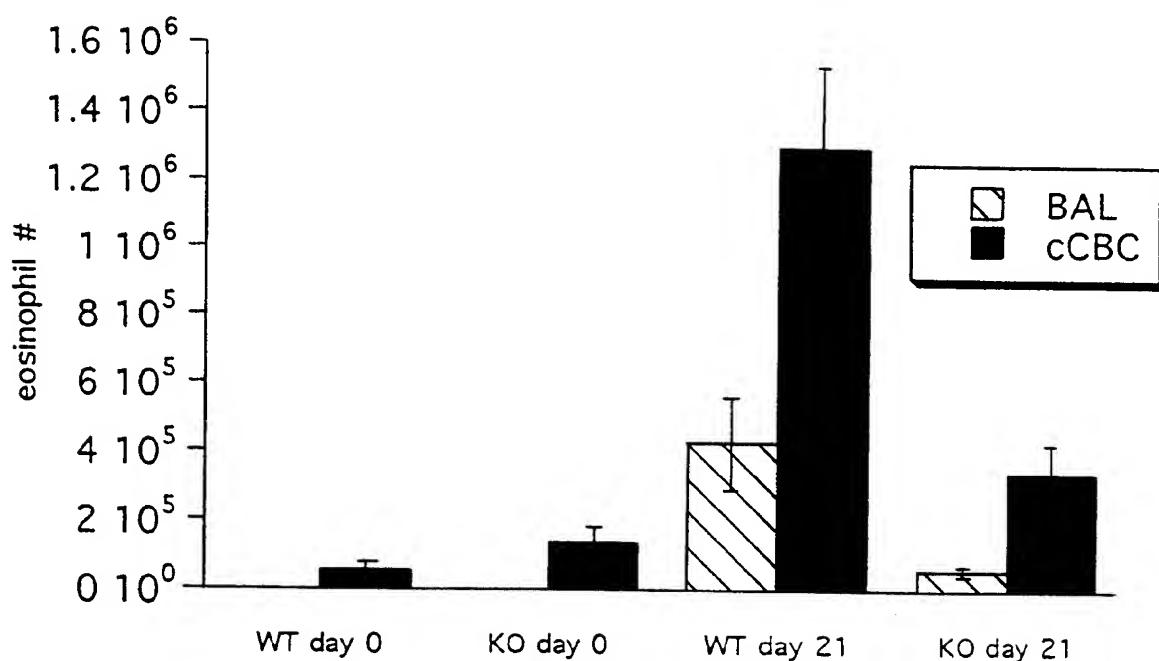
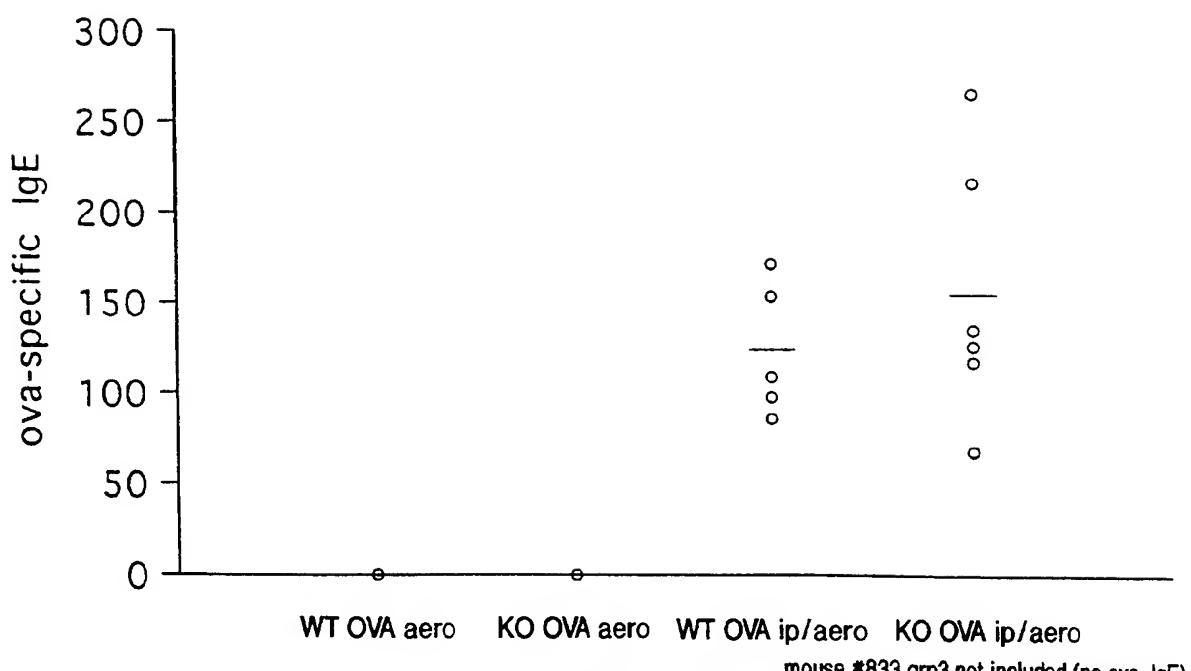


FIG. 31

FIG. 32
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FIG. 33

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FIG. 34

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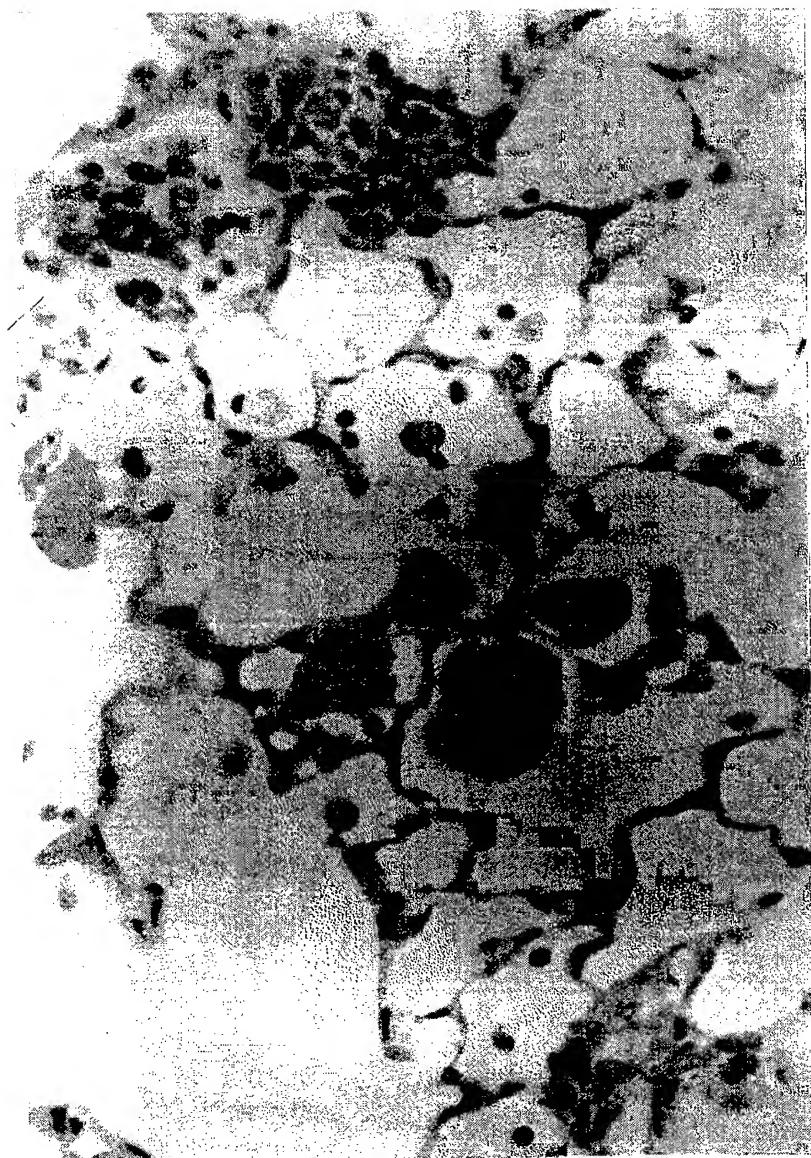


FIG. 35

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FIG. 36

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FIG. 37

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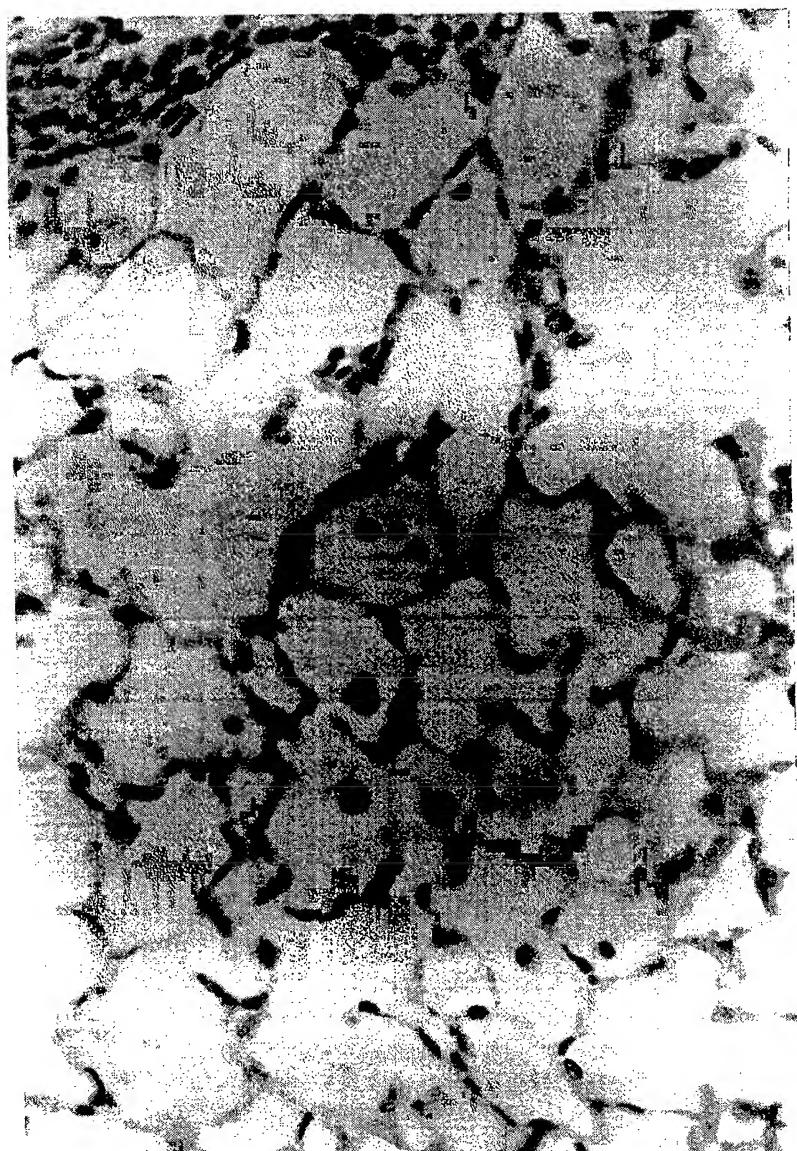


FIG. 38

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FIG. 39

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FIG. 40

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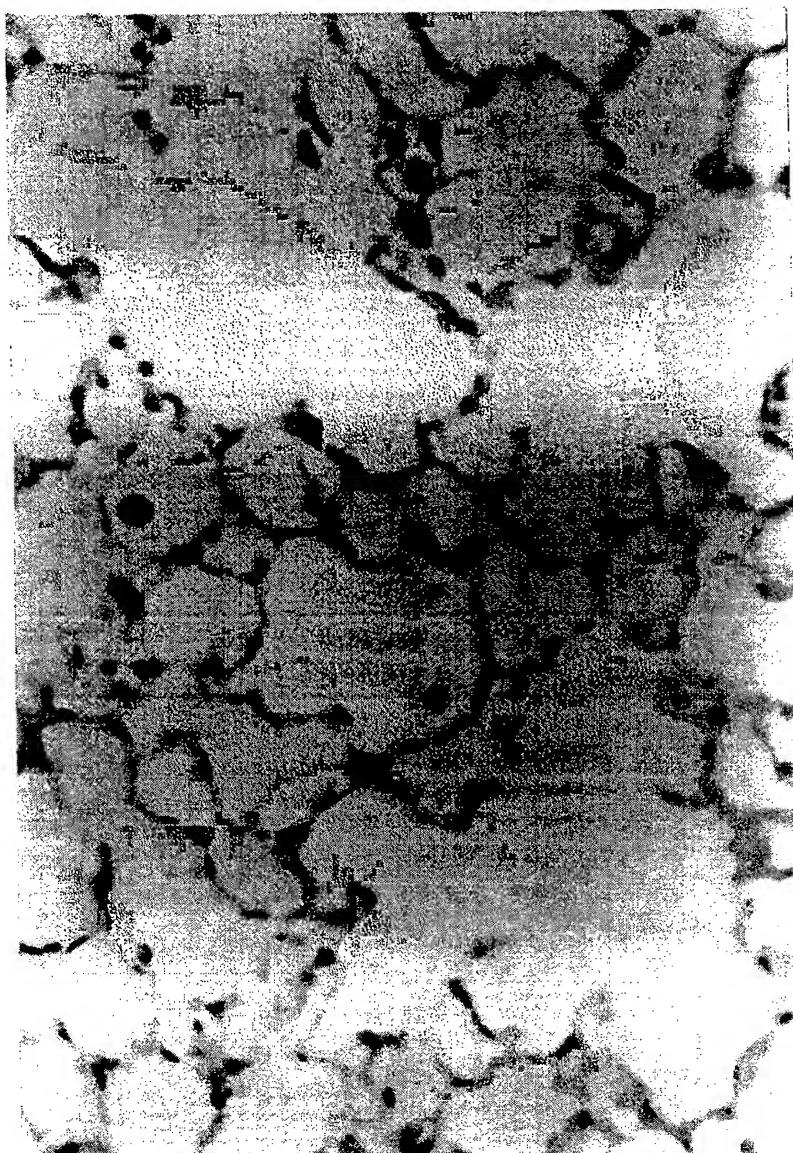


FIG. 41

INTERNATIONAL SEARCH REPORT

Int. Application No
PCT/US 96/11033

A. CLASSIFICATION OF SUBJECT MATTER
IPC 6 A61K39/395

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 6 A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

| Category | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

* Special categories of cited documents :

- 'A' document defining the general state of the art which is not considered to be of particular relevance
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- 'L' document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- 'O' document referring to an oral disclosure, use, exhibition or other means
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- '&' document member of the same patent family

Date of the actual completion of the international search

9 October 1996

Date of mailing of the international search report

05. 11. 96

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Fax (+31-70) 340-3016

Authorized officer

Olsen, L

INTERNATIONAL SEARCH REPORT

Int. Application No

PCT/US 96/11033

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

| Category * | Citation of document, with indication, where appropriate, of the relevant passages | Relevant to claim No. |
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International Application No

PCT/US 96/11033

| Patent document cited in search report | Publication date | Patent family member(s) | | Publication date |
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| | | EP-A- | 0550528 | 14-07-93 |
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